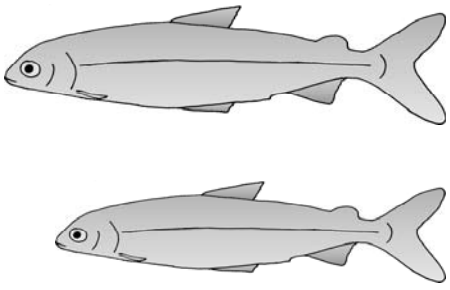
Eco-physiological and evolutionary divergence of a sympatric pair of coregonid fish



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Eco-physiological and evolutionary divergence of a sympatric pair of coregonid fish

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LIST OF PAPERS

This thesis is based on the five papers, which are referred to in the text by their roman numbers (I-V):

Paper I

J. Ohlberger, T. Mehner, G. Staaks and F. Hölker (2008). Is ecological segregation in a pair of sympatric coregonines supported by divergent feeding efficiencies? Canadian Journal of Fisheries and Aquatic Sciences 65: 2105-2113

Paper II

J. Ohlberger, G. Staaks and F. Hölker (2007). Effects of temperature, swimming speed and body mass on standard and active metabolic rate in vendace (*Coregonus albula*). Journal of Comparative Physiology B 177: 905-916

Paper III

J. Ohlberger, T. Mehner, G. Staaks and F. Hölker (2008). Temperature-related physiological adaptations promote ecological divergence in a sympatric species pair of temperate freshwater fish, *Coregonus spp.* Functional Ecology 22: 501-508

Paper IV

J. Ohlberger, G. Staaks, T. Petzoldt, T. Mehner and F. Hölker (2008). Physiological specialization by thermal adaptation drives ecological divergence in a sympatric fish species pair. Evolutionary Ecology Research 10: 1173-1185

Paper V

J. Ohlberger, Å. Brännström and U. Dieckmann (manuscript). A data-based model of evolutionary fish diversification through thermal adaptation along a temperature-depth gradient.

CONTENTS

ABSTRACT (ENGLISH)

The role of ecological factors in generating phenotypic and genetic diversity through natural selection has received increasing attention in evolutionary biology during the last decade, especially with respect to diversification in sympatry. Sympatrically occurring and closely related species are used as model systems to study the causes and mechanisms of ecological and evolutionary diversification. A sympatric pair of fish species coexists in the North-German Lake Stechlin: common vendace (*Coregonus albula*) and endemic Fontane cisco (*C. fontanae*). A speciation in sympatry within the lake has been suggested for these coregonids based on genetic analyses. Both species are morphologically similar planktivores with weak divergence in diet composition that co-occur within the pelagic area at slightly different water depths. Segregating mechanisms that facilitate ecological divergence and might have fostered the speciation were not known. Based on this knowledge, I investigated the eco-physiological and evolutionary divergence of this species pair. It was hypothesized that the species would differ in physiology and/or behaviour with respect to the most important environmental factors of their natural habitat. These factors are food density, light intensity and temperature. We studied the feeding efficiencies, metabolic rates, and temperature preferences of both species previously hatched and raised under identical laboratory conditions. Our results show that the divergence in feeding efficiency was insignificant for the most relevant environmental conditions. However, metabolic rates at various temperatures differed significantly between the species, suggesting that the deeper living Fontanae cisco is competitively superior at lower, but inferior at higher temperatures, compared to sympatric vendace. The conclusion that temperature-related physiological adaptations promote ecological divergence was supported by thermal preference tests showing that Fontanae cisco prefers significantly lower temperatures than vendace. The thermal preferences comply with temperatures of minimum net swimming costs, showing that performance is optimized at the respective thermal preference of the species. Hence, the use of slightly different thermal microhabitats within the pelagic area reduces exploitative competition and facilitates species' coexistence. This eco-physiological specialization along the vertical lake axis might have played a crucial role during sympatric speciation. To theoretically support this hypothesis, we developed a mathematical evolutionary model based on our field observations and laboratory experiments. The model showed that evolutionary splitting of an ancestral population into two populations with different temperature optima along the temperature-depth gradient is likely in this system. In conclusion, ecological and evolutionary diversification along the temperature-depth gradient is an empirically and theoretically plausible scenario for the sympatric speciation of the Lake Stechlin coregonids.

ABSTRACT (GERMAN)

Die Bedeutung ökologischer Faktoren bei der Entstehung phänotypischer sowie genetischer Vielfalt durch natürliche Selektion, besonders bei sympatrischer Artbildung, ist derzeit ein Fokus der Evolutionsforschung. Gemeinsam vorkommende und nah verwandte Arten werden daher als Modellorganismen verwendet, um die Ursachen und Mechanismen ökologischer und evolutionärer Diversifizierung zu untersuchen. Ein sympatrisches Fisch-Artenpaar existiert im norddeutschen Stechlinsee: die Kleine Maräne (*Coregonus albula*) und die endemische Fontane-Maräne (*C. fontanae*). Genanalysen deuten auf eine sympatrische Artbildung dieser morphologisch sehr ähnlichen Coregonen im Stechlinsee hin. Beide Arten sind rein planktivor, haben eine sehr ähnliche Nahrungszusammensetzung und kommen gemeinsam im Freiwasser in leicht unterschiedlichen Wassertiefen vor. Mechanismen, die zur ökologischen Segregation und damit zu einer beständigen Koexistenz beitragen, sowie die sympatrische Speziation bewirkt haben könnten, waren bisher nicht bekannt. Darauf basierend habe ich in meiner Doktorarbeit die öko-physiologische und evolutionäre Diversifizierung dieses Artenpaars untersucht. Die Hypothese war, dass sich die Physiologie bzw. das Verhalten der Arten im Bezug auf die wichtigsten Umweltfaktoren ihres Lebensraumes, Lichtintensität, Futterdichte und Temperatur, unterscheiden würden. Dazu haben wir Fraßeffizienz, Stoffwechselraten und Temperaturpräferenzen beider Arten untersucht, nachdem diese zuvor unter identischen Laborbedingungen herangezogen worden waren. Die Fraßeffizienz unterschied sich nicht signifikant im Bereich relevanter Umweltbedingungen. Allerdings zeigten beide Arten einen temperaturbedingt unterschiedlichen Stoffwechsel, was für die tiefer vorkommende Fontane-Maräne auf einen Vorteil bei niedrigeren Temperaturen, aber auf einen Konkurrenznachteil bei höheren Temperaturen hindeutete. Die Folgerung, dass temperaturbedingte Adaptationen die ökologische Diversifizierung fördern, wurde durch Verhaltensexperimente zu den thermischen Präferenzen der Tiere bestätigt. Die tiefer lebende Fontane-Maräne bevorzugt niedrigere Temperaturen als die Kleine Maräne. Die Temperaturpräferenzen entsprechen zudem den Temperaturen niedrigster Schwimmkosten, was wiederum auf eine Optimierung der Schwimmleistung in diesem Bereich hindeutet. Daraus folgt, dass die beiden Arten unterschiedliche Mikrohabitate nutzen, was die zwischenartliche Konkurrenz verringert und eine gemeinsame Existenz ermöglicht. Diese öko-physiologische Spezialisierung entlang der Vertikalachse des Sees könnte zudem ein entscheidender Faktor bei der Artbildung gewesen sein. Um diese Hypothese theoretisch zu stützen, haben wir basierend auf den Freiland- und Labordaten ein mathematisches Evolutionsmodell entwickelt. Demnach ist ein Aufspalten einer Ausgangspopulation in zwei Populationen mit unterschiedlichen Temperaturoptima wahrscheinlich. Eine ökologische und evolutionäre Diversifizierung entlang des Temperaturgradienten ist somit ein empirisch und theoretisch plausibles Szenario für die sympatrische Artbildung der Stechlinsee-Maränen.

1 BACKGROUND

1.1 Natural selection and adaptive speciation

> 'Natural selection, also, leads to divergence of character; for more living beings can be supported on the same area the more they diverge in structure, habits, and constitution…' (Darwin 1859)

A central question in evolutionary biology is how the stunning biological diversity on earth came about. The question dates back to Charles Darwin and his seminal book about the origin of species by means of natural selection (Darwin 1859). Darwin introduced the idea that populations evolve over the course of generations through the process of natural selection, which ultimately leads to evolutionary divergence. After being widely neglected during the late 19th and early 20th century, his ideas were finally rediscovered in the mid-20th century due to an increasing amount of theoretical and empirical evidence for natural selection and its fundamental role for evolutionary processes by biologists like Dobzhansky (1937), Mayr (1942), Lack (1947) and Simpson (1953). The modern theory of adaptive speciation and radiation is in line with this tradition of evolutionary thinking that micro-evolutionary processes driven by natural selection may finally lead to macro-evolutionary phenomena of diversification (Orr & Smith 1998; Schluter 2000; Coyne & Orr 2004; Dieckmann et al. 2004). It states that phenotypic and ecological diversity are ultimately the outcome of divergent or disruptive natural selection arising from differential resource use, competitive intraspecific interactions and ecological opportunity. Of major importance for the theory is that intraspecific competition can be frequency-dependent, which means that the selection pressures acting on a population depend on its phenotypic composition. Accordingly, empirical studies have confirmed that intraspecific competition can be frequency-dependent (Swanson et al. 2003; Schluter 2003) and it has been demonstrated that competition can cause ecological diversification within natural populations (Svanbäck & Bolnick 2007).

The idea that natural selection acting on competition for resources or habitats causes speciation, as already favoured by Darwin (1859), requires by definition 'sympatry', i.e. the absence of geographic barriers among diverging populations. In contrast, the architects of the neo-Darwinian synthesis reasoned that populations must be geographically isolated, i.e. in 'allopatry', for speciation to occur (Dobzhansky 1937; Mayr 1942). Otherwise, populations would be homogenized by gene flow, which prevents reproductive isolation. This view was later challenged by both theory and empirical data (Maynard Smith 1966; Bush 1969; Felsenstein 1981; Rice & Hostert 1993). Today, there is a lot of enthusiasm in favour of sympatric speciation and models support that it is theoretically possible and plausible, even despite high levels of gene

flow (Dieckmann & Doebeli 1999; Kondrashov & Kondrashov 1999; Doebeli & Dieckmann 2003; Gavrilets 2004). The most convincing empirical examples for sympatric speciation come from isolated environments like oceanic islands (Savolainen et al. 2006), crater lakes (Schliewen et al. 1994; Barluenga et al. 2006) or postglacial lakes (Gislason et al. 1999; Lu et al. 2001; Knudsen et al. 2006). However, empirical evidence is still scarce and the controversy whether species can arise in sympatry and how common this process might be in nature continues up to date (Jiggins 2006; Ortiz-Barrientos & Rieseberg 2006; Bolnick & Fitzpatrick 2007, Räsänen & Hendry 2008).

Empirical evidence is in fact difficult to gain since the speciation history cannot directly be studied. Therefore it is fundamental to investigate the specific traits that may be exposed to divergent natural selection and the ecological conditions that promote diversification of these traits (Coyne & Orr 2004). By studying the ecology, physiology and behaviour of closely related species, we cannot directly infer mode of speciation, but we might detect traits or characters under selection and elucidate the ecological forces shaping processes of ecological and evolutionary diversification. This is crucial to evaluate ecology's role in adaptive evolutionary processes.

1.2 The ecology of speciation and coexistence

> 'Although I do not doubt that isolation is of considerable importance in the production of new species, on the whole I am inclined to believe that largeness of area is of more importance…' (Darwin, 1859)

Not only whether species can arise in sympatry is under debate in evolutionary biology, but also whether one should continue to distinguish sympatric and allopatric speciation modes or rather concentrate on the mechanisms by which new species evolve. The mechanisms of ecologically based sympatric and allopatric speciation appear to be strikingly similar (see Schluter 2000). In both, disruptive or divergent natural selection is the driving force and the evolution of reproductive isolation is enhanced when ecological traits under selection lead to assortative mating (e.g. Dieckmann & Doebeli 1999; Bürger et al. 2006) or are correlated with other traits that cause assortment (e.g. location or timing of mating: Fry 2003; Friesen et al. 2007). 'Ecological speciation' is a concept that unites speciation processes, might it be in sympatry or in alloparty, with divergent natural selection on traits between environments and competition for resources as the two main processes driving phenotypic diversification and ultimately speciation. Laboratory experiments have shown that reproductive isolation has evolved as a by-product of adaptation to different environments in manipulative experiments (Rice & Hostert 1993) and there is also convincing evidence for its operation in nature (Schluter 2001). Furthermore, ecological specialization is the most important driving force for the evolution of reproductive isolation in theoretical models on sympatric

speciation (Doebeli & Dieckmann 2003; Dieckmann & Doebeli 2004; Rundle & Schluter 2004). Hence, biotic and abiotic environmental factors are potential forces for shaping patterns of diversification and their characteristics determine the strength of selection acting on phenotypic evolution and speciation (Losos et al. 1998; MacLean & Bell 2003; Kawecki & Ebert, 2004; Langerhans & Dewitt 2004).

The process of ecologically based speciation in sympatry by disruptive natural selection does not only require reproductive isolation between diverging populations, but it simultaneously requires the ability of the divergent populations to coexist (Coyne & Orr 2004; Gavrilets 2004). It is often the same ecological conditions allowing coexistence of different species that produce the kind of disruptive selection which is ultimately responsible for speciation. The 'competitive exclusion principle' predicts the outcome of interspecific competition as elimination or extinction of one of two species or populations that co-occur without niche differentiation (Gause 1934; Hardin 1960; Tilman 1982; Pianka 2000; Webb et al. 2002; but see also Darlington 1972). Consequently, closely related species that co-occur within the same habitat need to specialize in ecology, physiology or behaviour to avoid competitive exclusion and enable a persistent coexistence. This is often achieved by specialization along the diet, time or habitat niche axes (Robinson & Wilson 1994; Schluter 1996).

1.3 Environmental conditions

> 'It is notorious that each species is adapted to the climate of its own home: species from an arctic or even from a temperate region cannot endure a tropical climate, or conversely…' (Darwin, 1859)

The habitat selection and life-history of fishes is influenced by many interacting environmental parameters. Environment refers to biotic and abiotic elements of habitat as well as inter- and intraspecific interactions. Important abiotic factors are for example water temperature, light intensity, and oxygen content, whereas predation, food availability and competition are the major biotic factors (Clark & Levy 1988; Werner & Hall 1988; Becker & Eckmann 1992; Milinski 1993). Three of the major environmental resources for freshwater fishes in lake ecosystems are food, light and temperature. Food abundance and structure are critical parameters determining fish growth, reproduction and foraging behaviour. Light intensity is important because it enables visual predation, which affects the predator and prey interactions of planktivorous fishes. Fish vision is generally well adapted to the light characteristics of the environment and most planktivores highly depend on vision to catch prey (Janssen 1978; Lythgoe 1979; Gurthrie & Muntz 1993). The amount of light penetrating a water body further determines the temperature profile by providing energy for heating the water and the spatial distribution of primary production as a food resource for higher trophic levels.

Temperature is a critically important factor because of its fundamental effect on all biochemical processes (Wootton 1998). Especially poikilothermic animals like small fishes usually have a body temperature that matches the ambient temperature of their immediate surrounding medium. This has profound effects on individual performance and fitness (Huey & Berrigan, 2001), since most fishes adjust their behaviour to control body temperature within a species-specific range to optimize physiological processes and minimize disadvantageous temperature effects (Freidenburg & Skelly, 2004). Accordingly, in thermally heterogeneous environments, fishes select a temperature where physiological function is relatively efficient (Beitinger & Fitzpatrick, 1979; Angilletta et al., 2006).

One of the most important temperature-related traits in fishes is the foraging or swimming capacity, which largely determines success in food capture, reproduction, and predator avoidance (Videler 1993; Plaut 2001). The swimming physiology is thus presumed to be subjected to selection pressures that enhance evolutionary fitness (Reidy et al. 2000; Arnott et al. 2006). Accordingly, differences in metabolic rates between populations of one species may reflect adaptations to specific environmental conditions (Álvarez et al. 2006). This has immediate consequences for the competitive interactions between sympatrically occurring species or populations in thermally heterogeneous environments. It has been suggested that the existence of vertical gradients in water temperature may directly influence the competitive strengths of fish populations (Jensen et al. 2006). Moreover, temperature, not food or energetic potential, primarily determines the habitat choice in fishes (Garner et al. 1998; Krause et al. 1998; Wildhaber 2001). The behavioural trade-off between temperature, food availability and predation risk is of particular importance in patchy environments or those with relatively steep ecological gradients. Such environments are found in deep temperate lakes of the northern hemisphere, in which water temperature as well as zooplankton density and predatory fish are highly structured with regard to depth.

1.4 Sympatric pairs of freshwater fishes

> 'For it should be remembered that the competition will generally be most severe between those forms which are most nearly related to each other in habits, constitution, and structure…' (Darwin 1859)

Temperate freshwater fishes occupying postglacial environments are model systems for the study of adaptive diversification, because several taxa have generated species and ecological diversity in a manner consistent with the theory of adaptive speciation (Schluter 1996, 2000). These fishes are also predestined to study the role of ecological forces in causing evolutionary divergence, because most postglacially colonized lakes are small isolated and depauperate systems that offer ecological opportunities to occupy

niches where potential competitors or predators are absent. There is increasing evidence that ecological opportunity in species-poor postglacial lakes promotes adaptive divergence in polymorphic fishes in combination with relatively high intraspecific competition within the ancestral populations (Lu & Bernatchez 1999; Robinson et al. 2000; Vamosi 2003; Bolnick 2004; McKinnon et al. 2004).

Sympatric species pairs have been described in several fish groups from temperate regions of the Northern Hemisphere (see Taylor 1999). Examples come from sticklebacks (*Gasterosteus* spp.), smelts (*Osmerus* spp.), charrs (*Salvelinus* spp.) as well as whitefishes and ciscoes (*Coregonus* spp.). Some of these species pairs provide evidence for the process of sympatric speciation (e.g. Gislason et al. 1999; Knudsen et al. 2006), mostly driven by competition for resources or habitats (see also Robinson & Wilson 1994; Schluter 1996). There are many examples of sympatric fish pairs and most of them tend to divide resources and habitat in the same way. The common situation in these systems is the occurrence of limnetic-benthic species pairs that diverged into distinct ecotypes or species by exploiting either benthic food in profundal or littoral habitats, or planktonic food in pelagic habitats (Schluter & McPhail 1993, Lu & Bernatchez 1999; McKinnon & Rundle 2002; Amundsen et al. 2004; Kahilainen & Østbye 2006; Knudsen et al. 2006). This divergence is called trophic polymorphism and is thought to be a response to competitive pressures within a single ancestral population (Robinson et al. 1993; Skulason & Smith 1995). In contrast to systems displaying trophic polymorphism, species pairs that do not diverge in diet also co-occur (e.g. Smith & Todd 1984; Turgeon et al. 1999). In these cases, alternative segregating strategies that contribute to coexistence have not been described so far.

Coregoninae as a sub-family of the Salmonidae family have been subject of intense research efforts in evolutionary ecology because of their high phenotypic diversity and the co-occurrence of sympatric forms of divergent and reproductively isolated populations throughout the northern hemisphere (Taylor 1999). These fishes have undergone extensive radiation since the last glaciation period and species flocks as well as sympatric pairs have evolved in many taxa in the group, including the genus *Coregonus* (Behnke 1972; Svärdson 1979; Smith & Todd 1984). The distinct ecotypes and species most often vary in shape, growth rate, and body size, feeding preference or habitat selection (see Hudson et al. 2007). A great deal of the research on coregonines has investigated ecological and morphological differences between sympatrically occurring species, whereas only few studies exist that incorporate physiological measurements (e.g. Rogers et al. 2002). It appears that the sympatric speciation mode may not be uncommon in coregonines and some cases of collapses of several lineages into a single panmictic population suggest a prevalent role of ecology in coregonine speciation processes (Hudson et al. 2007). It is clear, however, that the modes of

speciation vary considerably between the different lineages and geographic areas. Sympatric pairs of vendace (*Coregonus albula*) and a coexisting sister species occur in several systems in the Baltic region, for instance in Finland (Vuorinen et al. 1981), Russia (Sendek 2002) and Germany (Schulz & Freyhof 2003; Schulz et al. 2006). In the North-German Lake Breiter Luzin and Lake Stechlin, autumn-spawning vendace populations coexist in sympatry with a spring-spawning, dwarf-sized sister species. The parallel evolution of the spring-spawning populations in both lakes by sympatric speciation has been suggested based on genetic analyses (Schulz et al. 2006). The species pair of Lake Stechlin was the study object for my work.

1.5 The study system

Lake Stechlin is a postglacial meso-oligotrophic lake in the northeast of Germany (53°10'N; 13°02'E). The lake was formed 12,000 years ago during the last glaciation period. It features no surface in- or outflows, has a mean depth of 22.8 m, a maximum depth of 68.5 m and a surface area of 4.25 km^2. The dominant pelagic fish species in Lake Stechlin is vendace, *Coregonus albula* (L.), which lives in sympatry with the endemic dwarf-sized Fontane cisco, *Coregonus fontanae* (Schulz & Freyhof 2003). The two species are easily distinguished by differential spawning times. Vendace spawns during winter (mid-December to mid-January), whereas Fontanae cisco spawns from spring to summer (end-March to mid-July). The species differ in some morphological characteristics, for instance in the number of scales in the lateral line, in body depth at dorsal fin origin and in interorbital distance of dorsal head length (Schulz & Freyhof 2003). The evolution of this species pair by sympatric speciation has been suggested from mtDNA and microsatellites analyses (Schulz et al. 2006) as well as AFLPs (K. Pohlmann, unpublished data). Both species perform regular diel vertical migrations by ascending from their deepwater daytime habitat into shallower water layers during nighttime (Mehner et al. 2007). However, the average nighttime population depths differ between the species, with Fontane cisco being found some meters deeper in the water column than vendace all over the year (Helland et al. 2007). This divergence in vertical distribution is associated with a difference in mean experienced water temperature (see Fig. 1). Despite the fact that the weak depth segregation within the pelagic area may facilitate a depth-related feeding divergence, diet compositions of both species are rather similar with a clear dominance of planktonic food. Consequently, trophic divergence does not contribute to competition avoidance and hence to the species' coexistence (Helland et al. 2008). This contrasts the commonly observed diversification along the limnetic-benthic axes found in many other sympatric freshwater fish pairs (Schluter & McPhail 1993, Lu & Bernatchez 1999; McKinnon & Rundle 2002; Amundsen et al. 2004; Kahilainen & Østbye 2006; Knudsen et al. 2006).

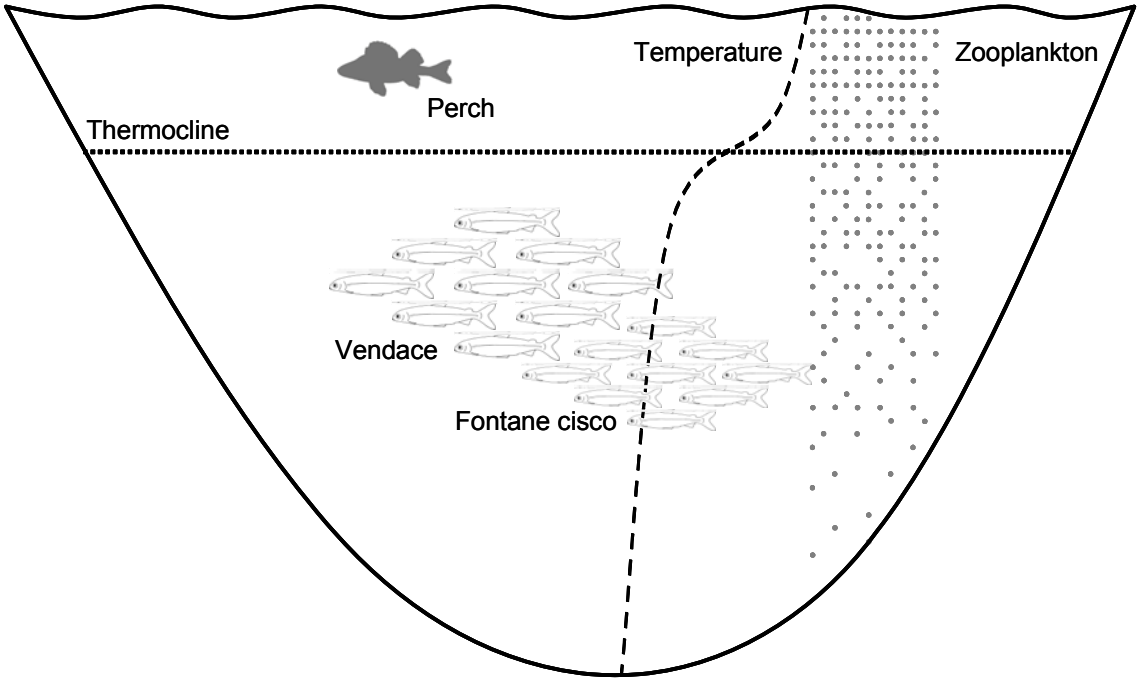

Fig. 1: Schematic illustration of the Lake Stechlin system. The pelagic and planktivorous coregonids inhabit the open water area of the lake below the thermocline, i.e. the hypolimnion, where they segregate ecologically along the temperature-depth axis. The resource density continuously declines with depth. Some piscivorous predators, mainly perch, are present in the epilimnion above the thermocline.

2 OBJECTIVES AND METHODS

The general objectives of my thesis were i) to detect environmental factors responsible for the ecological diversification of the Lake Stechlin coregonids, ii) to describe physiological and behavioural traits subjected to selection pressures, and iii) to infer potential forces and mechanisms that lead to the evolution of this species pair in sympatry. Recent investigations on present-day ecological segregation revealed a slight divergence of the two species along the temperature-depth axes of the pelagic environment (Helland et al. 2007), but no divergence in diet composition (Helland et al. 2008). This raised the question how the species reduce exploitative competition and persist in sympatry with such similar diet preferences and habitat characteristics. We therefore hypothesized that they might show physiological and/or behavioural differences with respect to the most determining biotic and abiotic factors of their natural habitat, food abundance, light intensity and water temperature.

2.1 Experimental approaches

Three main questions were posed with respect to a potential segregation of the species along the environmental gradients light intensity, food density and water temperature:

1. Do the species differ in their efficiency to consume zooplankton at various light intensities and food densities as an evolutionary adaptation to slightly different environmental conditions along the vertical lake axis?

To answer this question, we studied the foraging efficiency as a function of light intensity and food density of both species by performing functional response tests in the laboratory (**Paper I**). The fish were exposed to a range of naturally occurring food densities and light intensities to determine the relationship between prey density and consumption rate (Appendix: Fig. 2). Our assumption was that the deeper-living Fontane cisco is more efficient at low light levels and low prey densities which are typical for the deepwater areas, whereas vendace was expected to be superior at the brighter light and higher prey densities found in shallower water. Differential feeding efficiencies would reduce exploitative competition between the species by enhancing their competitive strength under the respective environmental conditions.

2. Do the species differ in the temperature-dependence of their standard and active metabolic rates as an evolutionary adaptation to slightly different thermal conditions?

To answer this question, we measured the metabolic rates of both species at various temperatures using respiration analyses in the laboratory (**Paper II, III**). We measured oxygen consumption rates in flow-through respirometers at temperatures to which the species are naturally exposed to compare the metabolic costs of living at different thermal regimes (Appendix: Figs. 3, 4). Our hypothesis was that the species might show

heritable trait differences in metabolic rate, i.e. physiological adaptations to the slightly differing microhabitats with respect to environmental temperature. Such a correspondence between field observations and experimental results could indicate how divergent natural selection may directly act on physiological traits thus contributing to ecological and possibly evolutionary divergence.

3. Do the species show different thermal preferences according to the slight divergence in vertical habitat use?

To answer this question, we determined the final thermal preferenda of the species (**Paper IV**). Our hypothesis was that they would behave differently in their temperature selection and that they would finally gravitate into different water temperatures in our experimental setup. We determined the thermal preferences by using a shuttle-box design where the fish were allowed to control body temperature over several weeks by altering the water temperature of the system (Appendix: Fig. 5). Our assumption was that the deeper-living Fontane cisco prefers cooler temperatures compared to sympatric vendace. A difference in thermal preference would indicate that thermal behaviour can facilitate ecological diversification and thereby reduce exploitative competition. A comparison with optimum temperatures for swimming served to evaluate whether thermal preference coincides with temperatures maximizing performance.

2.2 Theoretical approach

We further developed a data-based mathematical model of the evolutionary diversification of the coregonids in Lake Stechlin with the main question:

4. Is an evolutionary diversification of the coregonids along the temperature-depth gradient in the lake a theoretically plausible scenario for the speciation in sympatry?

The model we designed for this purpose was based on the adaptive dynamics theory, which has been used in many theoretical models investigating ecology-based processes of evolutionary diversification (**Paper V**). It assumes one quantitative adaptive trait (the optimum foraging temperature), a depth-dependent population dynamics with logistic competition for a single unstructured resource at each depth and an adaptive foraging behaviour of the fish along the temperature-depth axis according to food abundance, feeding conditions and predation risk. The main question was whether a system with a hypothetical ancestral population with any initial optimum temperature would experience evolutionary branching and a subsequent stable coexistence of two distinct morphs under the empirically derived parameter values and assumptions. Such an evolutionary splitting would indicate that the ecological conditions might have favoured a diversification of the Lake Stechlin coregonids and that a sympatric speciation along the temperature-depth axis is a theoretically plausible scenario.

3 MAIN RESULTS

In our experiments on the species' feeding behaviour we found only marginal divergence in capture rates between Fontane cisco and vendace (**Paper I**). In both species, capture rates decreased significantly with decreasing light intensity and increased with increasing prey density according to a type II functional response (i.e. at decreasing rate until satiation). Significant differences between the species were only found at the combination of highest light intensity and highest prey density, which represents a rather extreme and uncommon situation under natural condition. At all other light intensity and food density combinations, we observed no significant differences in their functional response. Thus, in contrast to our hypothesis, the ecological segregation between the co-occurring species is not facilitated by differences in feeding efficiency. Together with analyses of the species' diet compositions (Helland et al. 2008) these data suggested that the coregonids of Lake Stechlin are functionally similar planktivores without clear divergence in food use. Because of the ecological segregation along the vertical lake axis (Helland et al. 2007), where light intensity and food density decline continuously with depth (Mehner et al. 2005), the species are subjected to unequal consequences with respect to energy-budgeting. The deeper living Fontane cisco has to sustain energetic disadvantages compared to vendace, because it experiences lower food abundances and less favourable light conditions without showing any compensatory mechanism via enhanced feeding efficiency (**Paper I**). Although these disadvantages are partly compensated for by the general temperature effect on metabolic costs (i.e. lower costs in deeper and cooler water layers), this raised the question how the deeper living Fontane cisco is capable of sustaining a life-supporting energy budget under these conditions in direct competition with vendace. Alternative segregating mechanisms not related to feeding were suspected in order to facilitate coexistence between the species.

In our study on the swimming physiology of vendace (**Paper II**) we have used multivariate non-linear regression techniques to evaluate influences of body size, temperature and swimming speed on metabolic rates. This analysis showed that standard metabolic rates increased continuously with temperature, whereas total or net active metabolism did not. Further, vendace occurred to be very efficient in foraging, especially at intermediate temperature, with remarkably high optimum swimming speeds. Based on these analyses, we compared the maintenance and active metabolic costs of both species with respect to environmental temperature and foraging speed to determine whether temperature influences the species' metabolism differently (**Paper III**). We found that Fontane cisco has a reduced standard metabolic rate compared to vendace over the whole range of environmental temperatures. However, the net active metabolic rate was lower in Fontane cisco at 4°C, but higher at 8 and 15°C compared to

vendace. As a consequence, total metabolic costs when foraging at optimum speeds were higher in vendace at 4°C, but higher in Fontane cisco at 8°C or more (Fig. 6). This was in accordance with the ecological field surveys which indicated that the Fontane cisco population was not found in water layers with a temperature above approximately 8°C (Helland et al. 2007). Fontane cisco is thus better adapted to lower temperatures with respect to metabolism than vendace, suggesting that species-specific physiological adaptations contribute to ecological divergence and a stable coexistence of the species. Hence, besides the general temperature effect, a metabolic adaptation in Fontane cisco (**Paper III**) implies further compensation of the energetic disadvantages associated with living in deeper water (**Paper I**). Moreover, our results indicated that standard metabolism was under strong selection in Fontane cisco (**Paper III**), probably due to the relatively high maintenance compared to swimming costs in these fishes (**Paper II**). Since different physiological traits or cellular processes can be subjected to differential selection pressures, we were left in uncertainty whether the reported temperature-dependences of the active metabolic costs represented an optimizing strategy on the organism level and whether the species would prefer these temperatures accordingly.

Our temperature preference tests revealed significant differences in the species' temperature selection behaviour (**Paper IV**). The species-specific final thermal preferenda were 9.0°C for vendace and 4.2°C for Fontane cisco, indicating evolutionary cold adaptation in the deeper living species (Fig. 6). These results were in line with our study on the species' metabolic costs (**Paper III**) since Fontane cisco preferred a temperature where it is competitively superior with respect to metabolism, compared to vendace. In our study on the swimming physiology of vendace we had demonstrated that net swimming costs in this species showed no clear trend with temperature (**Paper II**). After re-evaluating the metabolic rates data for both species, it became evident that net costs of swimming are optimized at the range of thermal preference in both, Fontane cisco and vendace. Net swimming costs were lowest in Fontane cisco at 4°C and in vendace at 8°C (**Paper IV**). This correspondence of thermal preference and thermal physiology (Fig. 6), together with the vertical habitat segregation (Helland et al. 2007), finally showed that the species are adapted to different thermal regimes within their pelagic environment.

We had hypothesised that this thermal adaptation might have played an important role during the evolutionary diversification in sympatry, since it facilitates the ecological segregation observed in the system. The evolutionary, adaptive dynamics based model showed that the ecological conditions in Lake Stechlin may have led to evolutionary branching along the temperature-depth gradient (**Paper V**). In our model, a hypothetical ancestral population evolves to an evolutionary singular strategy, where it experiences a fitness minimum and thus disruptive selection on the adaptive trait (optimum foraging

temperature) leading to evolutionary branching. Subsequently, dimorphic evolution proceeds to a point where the selection gradient vanishes and two populations with divergent temperature optima and depth-distributions are established. The dimorphic system represents an evolutionary endpoint since it is not subjected to further branching processes. The model outcome resembles the real system remarkably well. The trait values of the model populations are similar to the measured temperature preferences and metabolic optima and the resulting depth distributions are in good accordance with the observed average population depths of the Lake Stechlin coregonids.

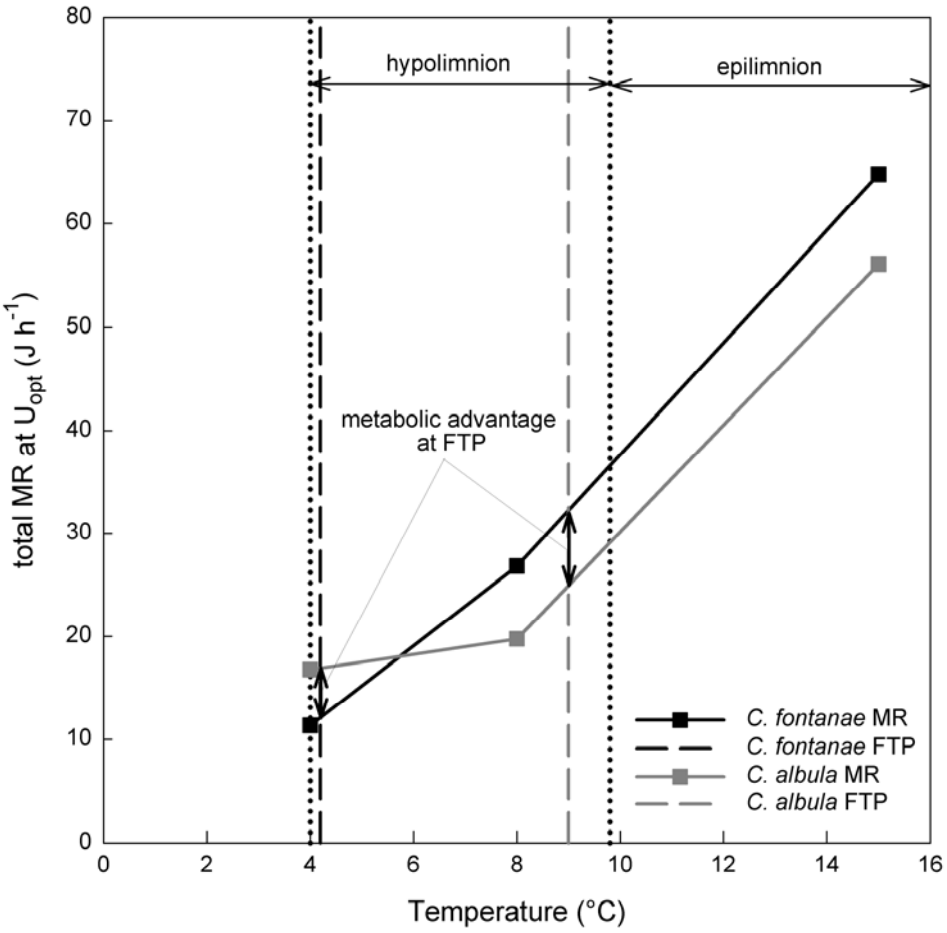

Fig. 6: Overview of the most important temperature-related physiological attributes of vendace (black) and Fontane cisco (grey). Shown are final thermal preferences (FTP, dashed lines) and the total metabolic rates (MR, squares and solid lines), which are calculated for a 20g fish swimming at optimum speed. The metabolic advantages of the species in comparison to each other at the respective thermal preferences are indicated by double-arrows. These make up about 30% of total energy use at optimum speed. The temperature range of the hypolimnion is indicated by the dotted lines for the bottom temperature (4°C) and the thermocline (9.8°C, averaged over all months of stratification).

4 CONCLUSIONS

4.1 Eco-physiological divergence

It has been shown that the sympatric species pair of Lake Stechlin displays an ecological segregation along the vertical axis of the pelagic environment (Helland et al. 2007). No significant differences were found with respect to feeding morphology (Schulz & Freyhof 2003), feeding preference (Helland et al. 2008) or feeding efficiency (**Paper I**). Both species are thus pelagic planktivores without clear divergence in food use. This contrasts the majority of other systems with sympatric fish pairs, where the common pattern is that of resource partitioning along the limnetic-benthic axes (e.g. Schluter & McPhail 1993; Gislason et al. 1999; McKinnon & Rundle 2002; Knudsen et al. 2006), as well as other systems with sympatrically occurring coregonids (Bernatchez et al. 1999; Lu & Bernatchez 1999; Amundsen et al. 2004; Kahilainen & Østbye 2006). Some studies have described a separation in vertical habitat use between closely related coregonids (Smith & Todd 1984; Gjelland et al. 2007). In these cases, however, segregating mechanisms other than divergence in depth distribution were not proposed.

With our finding that the Lake Stechlin coregonids show distinct temperature-related physiological adaptations (**Papers II, III**), we describe a novel mechanism of eco-physiological specialization of a sympatric pair of freshwater fish. The reported metabolic adaptations promote ecological divergence. This facilitates a stable coexistence, because the species achieve energetic advantages over the competitor within the respective thermal regime thereby avoiding competitive exclusion. This conclusion was corroborated by our analysis of the thermal preferences of both species which differ according to the ecological segregation and coincide with temperatures of minimum net swimming costs (**Paper IV**). Swimming performance is thus optimized at the respective preferred temperature (Fig. 6), indicating that thermal preference and thermal physiology are coadapted. Such a correspondence of thermal preference and temperatures maximizing performance has never been described so far for the divergence of closely related sympatric species. Based on these experimental results and the ecological field data (Helland et al. 2007; Mehner et al. 2007), it became evident that temperature, rather than food use or light regime, is the predominant environmental factor shaping the competitive interaction and ecological divergence of this species pair. Temperature mediated the eco-physiological specialization by allowing the species to occupy different microhabitats along the vertical lake axes. With our theoretical approach we were able to support these conclusions by showing that, irrespective of the evolutionary dynamics, a stable coexistence of two morphs with different optimum foraging temperatures is possible in this system when using our data-based assumptions and parameter functions (**Paper V**).

4.2 Temperature effects

It is widely accepted that fishes are usually well adapted to the environmental temperature of their natural habitat on small spatial scales (Magnuson et al., 1979; Johnson & Kelsch, 1998; Pörtner, 2002) and that the broad-scale biogeography of fishes is shaped by the direct effects of temperature as well (Murawski 1993, Pörtner et al. 2007). Accordingly, it has been suggested that adaptive differences in thermal physiology promote ecological divergence between intertidal animals (Somero 2002; Pulgar et al. 2005) and even macro-ecological and phylogeographic patterns in ectothermic animals may be explained by variations in temperature-related physiological attributes (Bernardo et al. 2007). Temperature differences between adjacent microhabitats are further believed to cause disruptive selection on various adaptive traits (e.g. Baker et al. 2005). Moreover, it has been shown that niche segregation of fishes can be facilitated by differentiation in thermal preference (Larsson 2005). The relationship between thermal physiology and interspecific differences in habitat use of fishes is nonetheless poorly understood.

Here, we present the first case of closely related species for which it is shown that temperature-related metabolic adaptations lead to ecological divergence by differential thermal microhabitat use (**Papers III and IV**). This segregating mechanism might be the cause of stable coexistence of other sympatrically occurring fishes for which differences in food do not seem to cause divergence. That might be the case, for instance, in the cisco populations of the North American Great Lakes (see Smith & Todd 1984; Turgeon et al. 1999) or the vendace and whitefish populations of the Scandinavian Pasvik watercourse (see Gjelland et al. 2007; Bohn et al. 2008). In the latter case, it has already been suggested that the use of different microhabitats along a lake's depth-gradient enables a persistent coexistence of native whitefish (*Coregonus lavaretus*) and invading vendace.

4.3 Ecologically based speciation

It is widely believed that the same ecological conditions that produce disruptive natural selection and cause adaptive speciation drive ecological differentiation and enable the coexistence of closely related species in sympatry (Schluter 2000; Coyne & Orr 2004). With our theoretical approach based on the adaptive dynamics theory, we showed that the same ecological conditions that are believed to facilitate a persistent coexistence of the two coregonids in Lake Stechlin are prone to evolutionary branching of a hypothetical ancestral population (**Paper V**). The model thus demonstrated that metabolic thermal adaptation along the temperature-depth gradient as a basis for evolutionary diversification is a theoretically feasible scenario. It is the first empirically motivated and data-based model showing the plausibility of a sympatric speciation

along a gradient in environmental temperature. Hence, the combination of i) the observed vertical segregation, ii) temperature-related metabolic adaptations, iii) the reported differences in thermal preference and iv) the evolutionary model, suggest the temperature-gradient as a driving environmental factor that fostered the evolutionary diversification of the Lake Stechlin coregonids.

Similar processes of ecologically based adaptive speciation along temperature gradients may have occurred in other systems of freshwater fishes. The idea is corroborated by general theoretical studies suggesting that speciation along environmental gradients may be a common process in nature (Doebeli & Dieckmann 2003). Moreover, other empirical studies have found that the effect of natural selection operating along ecological gradients can be sufficiently strong to overcome the homogenizing effect of gene flow and induce evolutionary novelty (e.g. Schneider et al. 1999). A growing body of evolutionary models nowadays favours sympatric speciation as a driving force in generating biological diversity (see Coyne & Orr 2004; Dieckmann et al. 2004; Kirkpatrick & Ravigne 2002; Bolnick & Fitzpatrick 2007) and the number of theoretical approaches that incorporate ecological gradients into the study of diversification are increasing (e.g. Mizera & Meszéna 2003; Leimar et al 2008).

Other factors, besides the presence of an environmental gradient, that have been proposed as pre-requisites for an ecologically based adaptive diversification of freshwater fishes are a species-poor environment and a relatively high intraspecific competition within the ancestral population (Lu & Bernatchez 1999; Robinson et al. 2000; Vamosi 2003; Bolnick 2004; McKinnon et al. 2004; Hudson et al. 2007). It can be assumed that these pre-requisites were present in Lake Stechlin after the last glaciation. At the time the lake was colonized by an ancestral coregonid population, it presumably was a species-poor environment with low productivity and thus high intraspecific competition within the ancestral population, but low numbers of predator species as well as competing planktivores within the pelagic area. Still today this lake holds only few other fish species than the coregonids, namely roach (*Rutilus rutilus*), perch (*Perca fluviatilis*) and bleak (*Alburnus alburnus*), and all of them are low in abundance (Mehner & Schulz 2002; Schulz et al. 2003). Further, biogeographical considerations support the conclusion that these system characteristics were important for the evolutionary diversification. Sympatric pairs of *Coregonus* spp. occur in only two lakes in Germany, Lake Stechlin and Lake Breiter Luzin (Schulz et al. 2006). These two lakes are the deepest in that region (Mehner et al. 2005) and both are located at the southern border of the vendace distribution range (Kottelat & Freyhof 2007). Consequently, these lakes have the broadest temperature ranges and the steepest gradients compared to other lakes where vendace occurs.

An alternative hypothesis about the species' evolution is sympatric speciation by temporal separation of spawning time, since the coregonids in Lake Stechlin spawn at different times of the year (Schulz & Freyhof 2003). Many sympatric populations of coregonids have temporal or spatial divergence in spawning (Kottelat & Freyhof, 2007). It has been demonstrated in other animal taxa that temporal separation of reproduction can sufficiently reduce gene flow between closely related sympatric species and cause sympatric speciation to occur (e.g. Friesen et al. 2007). However, the evolution of reproductive isolation is not sufficient to explain a persistent coexistence of species if habitat and food use is identical. Population density of the competitively inferior population would be expected to decrease to extinction as a consequence of competitive exclusion (Pianka 2000). Consequently, competition avoidance and hence coexistence has to be based on ecological divergence (Coyne & Orr 2004; Gavrilets 2004). Since we found surprisingly similar food use between the species, differential use of thermal microhabitats might be the only mechanism by which this is achieved. Whether the divergence in thermal microhabitat use caused a shift in spawning time cannot be answered based on the present work. A possible explanation for the shift is that bioenergetic constraints for individuals preferring lower temperatures delayed maturation thereby causing a switch from winter- to spring-spawning. In this case, selection on the thermal preference trait would indirectly cause assortment and consequently reproductive isolation between the species.

In conclusion, my thesis shows that the temperature-depth gradient of the lake mediates the eco-physiological divergence of this species pair suggesting this gradient as a driving force for the speciation in sympatry. The thesis thereby highlights the importance of temperature gradients for shaping processes of ecological and evolutionary diversification. This is a new perspective on the ecologically based evolution of closely related sympatric species, especially with respect to fish pairs that commonly display a divergence in food use or are believed to do so. The work further suggests that it might be advantageous to consider the adaptive potentials of species and their evolutionary history for applying successful protection plans for endangered freshwater fishes that are highly subjected to temperature changes, and for evaluating potential impacts of climate change on fish populations and communities.

5 PERSPECTIVES

To emphasize the significance of this work and to give some perspectives, suggestions for future research in this field are made (general). Further, a couple of questions with respect to the Lake Stechlin coregonids remain unsolved and appear to be promising research areas (specific). The latter are partly thought ahead by other researchers of our group (J. Freyhof, I.P. Helland; F. Hölker, T. Mehner, G. Staaks).

General

Studies on the ecological and evolutionary diversification of closely related fish species have rarely focused on physiological traits (but see Rogers et al. 2002). Their role thus needs further attention. Especially temperature-related physiological differences of sympatric fish pairs have never been studied so far, although adaptation to environmental temperature is a common phenomenon in poikilothermic animals. Thermal adaptation occurs at various levels of biological organisation, ranging from molecular to organismal. Nevertheless, knowledge about the detailed events that occur between the reception of ecological or evolutionary stimuli and the manifestation of the response in terms of physiological traits is scarce (see Feder et al. 2002; Clarke 2003) and functional links between genetic/molecular and ecological/evolutionary adaptive processes are barely understood (Pörtner et al. 2006). In order to obtain a mechanistic understanding of thermal metabolic adaptation, we need analyses of the genes involved and the functions of their products within the organism and use these to provide a link between the various levels of organisation. A functional understanding of thermal adaptation is further necessary to provide meaningful solutions to problems like biodiversity conservation and global climate change.

Specific

What are the impacts of climate change on the Lake Stechlin coregonids?

A predictive model simulating a future temperature increase could evaluate the adaptive response of the Lake Stechlin coregonids to an increase in water temperature. Such a model could be based on the presented evolutionary model (**Paper V**) and a previously developed model that predicts the impacts of temperature change on the lake's water body and plankton communities (Gerten & Adrian 2001). This approach would provide insights to the potential threats for the system resulting from future climate change.

Which life-history traits are involved in the divergence in spawning time?

Since we have no conclusive explanation for the divergence in spawning time, future research might use life-history traits to shed light on the question how temporal isolation in spawning, and consequently reproductive isolation, evolved. Different

spawning grounds or temperatures of hatching fry may have differential selective effect on the two species, thereby increasing the relative fitness of one or the other.

Are the species completely reproductively isolated?

Hybridization experiments could be used to determine whether the species can still interbreed with each other and produce fertile offspring. To perform such experiments, it is necessary to synchronize the species' maturation in the laboratory or fertilize mature eggs from one of the species with previously frozen spermatozoa from the other. If hybrids hatch, a fertility test with the offspring itself is needed. In case of unfertile offspring, the test would provide the final proof for their status as distinct species.

To what degree is microhabitat selection a genetically fixed or ontogenetically learned behaviour?

Tagging experiments with laboratory fish of both species raised under the same conditions that are released into Lake Stechlin could be used to determine whether thermal habitat use in these fishes is a fully heritable behaviour or influenced by ontogenetic effects. Similar behaviour of laboratory raised and wild fish of the same species should be observed if microhabitat selection is mainly determined by genetic effects, whereas similar behaviour of laboratory fish of both species would emphasise the ontogenetic influence on habitat selection.

Are bioenergetic constraints responsible for the shift in spawning time?

Bioenergetic analyses and individual-based modelling could be used to identify whether thermal adaptation with its immediate energetic consequences can explain a shift in spawning time due to a delayed maturation of those phenotypes that prefer lower temperatures.

Which biochemical processes are involved in thermal metabolic adaptation?

To study the sequences (amino acids), concentrations (gene expression, mRNA) and activities (milieu) of enzymes that are involved in temperature adaptation, for instance lactate dehydrogenase (LDH), might be helpful to increase the functional understanding of the quantitative processes involved (see Somero 2004).

What is the genetic basis for the thermal metabolic adaptation?

Genome screening for DNA polymorphisms might elucidate the genetic basis of the adaptive differences between these closely related species, since it is likely that the selective regimes that caused disruptive selection produced different quantitative trait loci (Storz 2005). Hence, if specific target genes can be identified, a genome screening may detect the locus-specific signatures, which can then be related to phenotypic divergence, thereby providing a causal link between genotype and phenotype.

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DECLARATION OF AUTHORSHIP

English version

I hereby declare that this PhD thesis has been written only by the undersigned, without any assistance from third parties and that no sources other than those indicated have been used. This thesis has not been submitted for a doctor's degree at any other institution and I am not holding a corresponding doctor's degree. I am aware of the underlying doctorate regulations of the faculty this thesis is submitted to, Faculty of Agriculture and Horticulture of the Humboldt-University at Berlin.

German version

Hiermit erkläre ich, die Dissertation selbständig und nur unter Verwendung der angegebenen Hilfen und Hilfsmittel angefertigt zu haben. Ich habe mich nicht anderwärts als Doktorand beworben und besitze keinen entsprechenden Doktorgrad. Ich erkläre die Kenntnisnahme der dem Verfahren zugrunde liegenden Promotionsordnung der Landwirtschaftlich-Gärtnerischen Fakultät der Humboldt-Universität zu Berlin.

Berlin, 15.10.2008

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Jan Ohlberger

APPENDIX

Figures

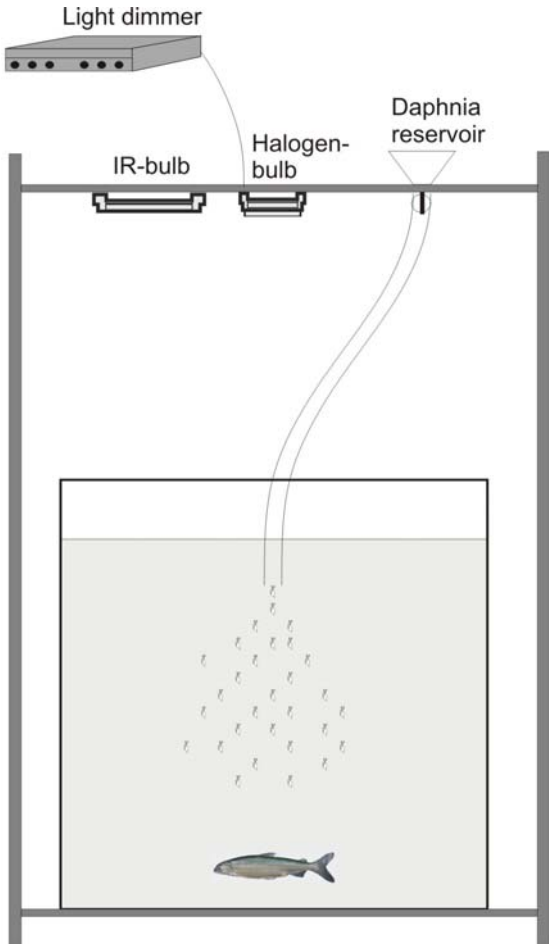

Fig. 2: Functional response test for capture rate determination. Feeding behaviour was observed in glass aquaria placed in a climatic chamber which was darkened with light-impermeable curtains. Luminaries equipped with halogen bulbs connected to a digital/analogue dimmer were installed above the aquaria. Daphnia were introduced through a tube from a reservoir above the aquaria. Additionally, infrared lamps were installed to allow observation of the feeding activity at very low light intensities using night-vision glasses.

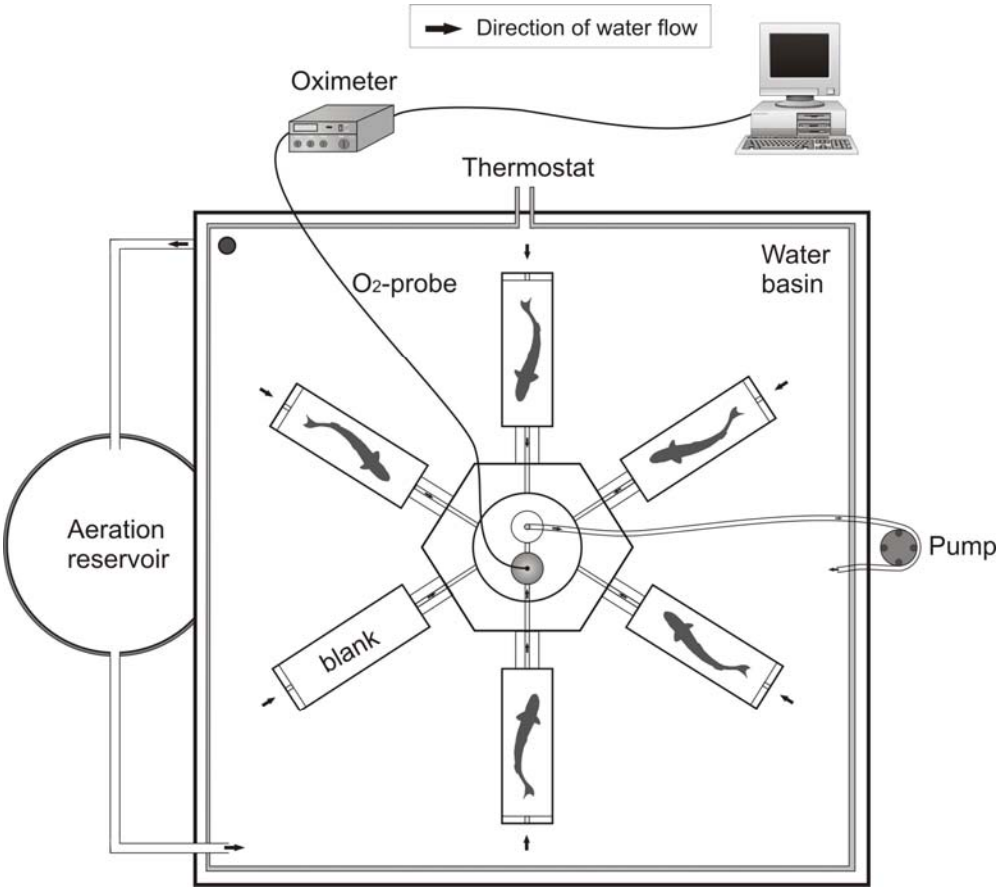

Fig. 3: Flow through respirometer for standard metabolic rate determination. Five fish were placed in Plexiglas chambers for measurements of metabolic rates using a flow through system driven by a tubing pump. The sixth chamber was used to determine microbial respiration. The oxygen content of the water was measured using an oxygen probe connected to an oxymeter which transferred the data to a notebook for subsequent processing.

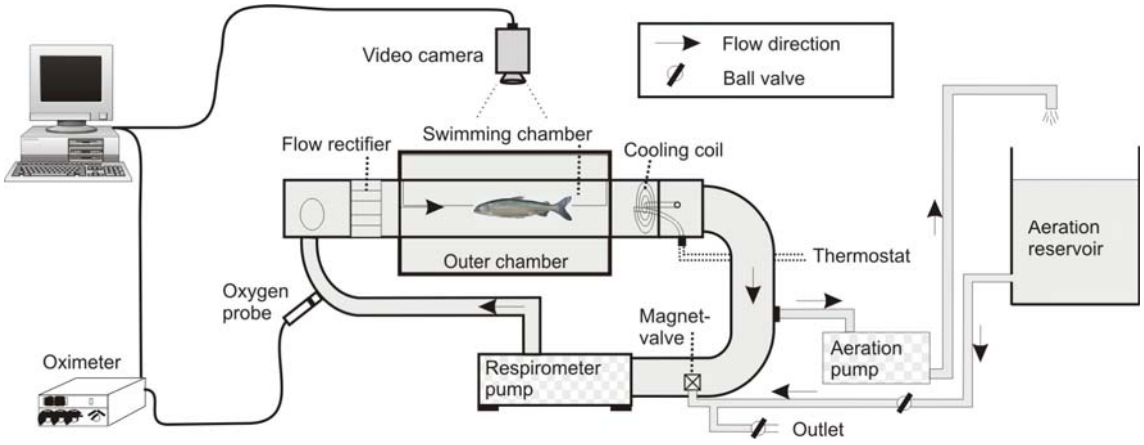

Fig. 4: Intermittent-flow through swim tunnel for active metabolic rate determination. Fish were forced to swim against a water current of a given speed generated by a rotary pump. During swimming the oxygen consumption of the fish was measured continuously with a fixed oxygen probe and recorded by a computer. The oximeter allowed automated flushing and measuring by opening and closing a ventilation cycle via a magnet valve. During flushing the preset oxygen content of the measuring unit was re-established. A video camera was used for monitoring the fish during experiments (modified from Ohlberger et al. 2005).

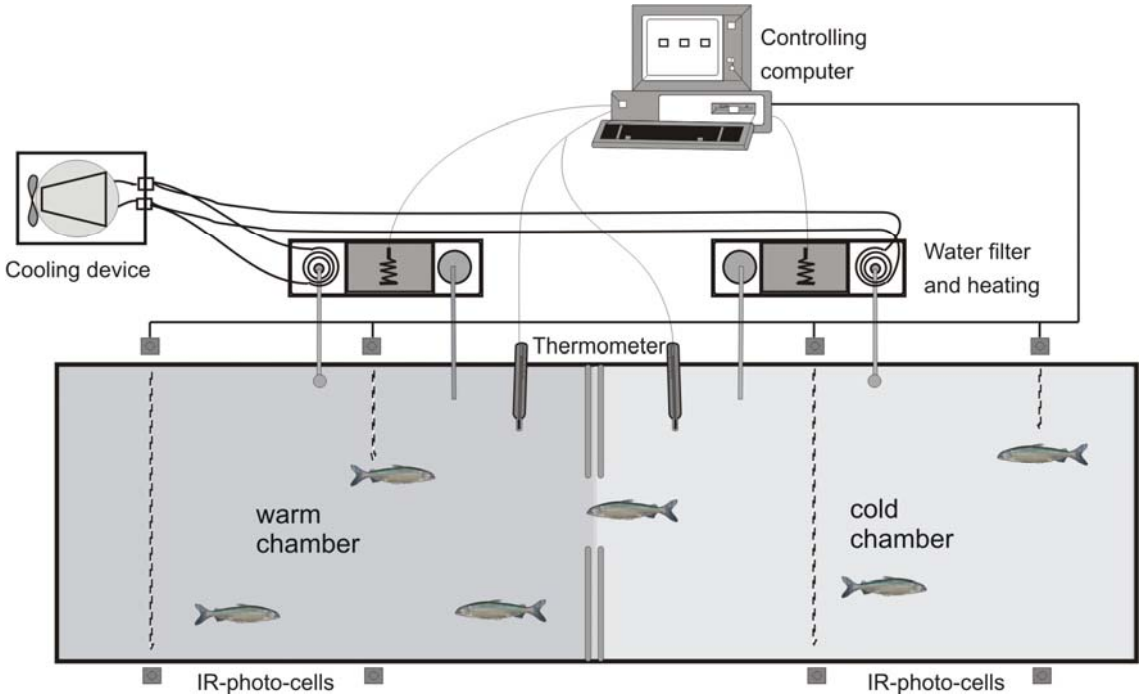

Fig. 5: Shuttle box system for temperature preference determination. Three fish were placed in each of the two interconnected compartments of an aquarium separated by a plastic panel with a centred hole. After acclimation, a constant temperature difference of 2 Kelvin was established between the sections by cooling coils and electric heaters, which were controlled by a computer. Infrared transmitters and photocells continuously monitored the activity of the fish within the sections. The system was automatically cooled down or heated up in a dynamic manner according to the distribution of the fish.

Paper I

I

Is ecological segregation in a pair of sympatric coregonines supported by divergent feeding efficiencies?

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Is ecological segregation in a pair of sympatric coregonines supported by divergent feeding efficiencies?

Jan Ohlberger, Thomas Mehner, Georg Staaks, and Franz Hölker

Abstract: Some of the sympatric species pairs commonly described in temperate freshwater fishes provide evidence for ecological specialization driven by competition for food resources as a potential prerequisite of subsequent sympatric speciation. In the postglacial Lake Stechlin (Germany), two sympatric coregonines coexist, common vendace (*Coregonus albula*) and endemic dwarf-sized Fontane cisco (*Coregonus fontanae*). The species segregate vertically along the light intensity and prey density gradients of their pelagic environment. Accordingly, we hypothesized that the species might show differences in their foraging efficiency associated with these environmental gradients. We investigated the feeding behaviour by measuring the functional response of both species to *Daphnia magna* at various prey densities (0.25–8 individuals·L^{-1}) and light intensities (0.005–5 lx) at a deep blue light spectrum to simulate their natural habitat. Decreasing light intensity and prey density significantly depressed consumption rates in both species. Overall, we observed only weak differences in feeding behaviour, which indicates that the species are functionally similar, coexisting planktivores.

Résumé : Quelques-unes des paires d'espèces sympatriques couramment décrites chez les poissons d'eau douce des régions tempérées montrent des signes de spécialisation écologique reliée à la compétition pour les ressources alimentaires, ce qui est potentiellement une condition préalable pour une spéciation sympatrique subséquente. Dans le lac Stechlin (Allemagne), un lac post-glaciaire, coexistent deux corégoninés sympatriques, le corégone blanc (*Coregonus albula*) et le corégone de Fontane (*Coregonus fontanae*), une espèce endémique naine. Il y a ségrégation verticale des espèces le long des gradients d'intensité lumineuse et de densité de proies dans leur milieu pélagique. C'est pourquoi, nous formulons l'hypothèse selon laquelle les espèces devraient montrer des différences d'efficacité de recherche de nourriture en relation avec ces gradients environnementaux. Nous avons étudié leur comportement alimentaire en mesurant la réponse fonctionnelle des deux espèces à *Daphnia magna* à diverses densités de proies (0,25–8 individuals·L^{-1}) et sous différentes intensités lumineuses (0,005–5 lx) dans un spectre de lumière bleu foncé afin de simuler leur habitat naturel. Une intensité lumineuse et une densité de proies décroissantes réduisent les taux de consommation chez les deux espèces. Globalement, nous n'observons que de légères différences de comportement alimentaire, ce qui indique que les espèces sont des planctonophages qui coexistent tout en affichant un fonctionnement similaire.

[Traduit par la Rédaction]

Introduction

Sympatric species pairs have been most commonly described in several families of teleost fishes in temperate postglacial lakes, basically in sticklebacks, smelts, and salmonids, including the coregonines (Taylor 1999). Many of these species pairs show clear ecological segregation (Schluter 1996, 2000), wherefore much of the recent research in this field has focused on the role of ecological specialization in driving and maintaining divergence in sympatry. There is ample evidence that the process is often driven by competition for food resources (Robinson and Wilson 1994; Smith and Skúlason 1996), and evidence for the process of sympatric speciation has been provided for some of these species pairs (e.g., Gislason et al. 1999; Knudsen et al. 2006).

Sympatrically living species are common among coregonines (whitefishes and ciscoes; see Hudson et al. 2007). Many of them exhibit profound differences in feeding strategies and habitat preferences (e.g., Lu and Bernatchez 1999; Amundsen et al. 2004). However, species pairs that do not display a divergence in diet or habitat also co-occur, for instance in the North American Lake Superior (Smith and Todd 1984; Turgeon et al. 1999). In these cases, alternative segregating strategies that contribute to the coexistence of the species have not been described so far. Since many environmental factors such as temperature, turbidity, and especially the light regime influence the feeding behaviour of

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planktivorous fish (Matthews 1998; Wootton 1998), diverse feeding efficiencies of sympatric planktivorous species might be expected if the species segregate along environmental gradients.

It is generally assumed that fish vision is well adapted to the light characteristics of the environment (Lythgoe 1979), and spectral adaptations have been described in many deepwater marine fishes (Lythgoe 1988). Planktivorous coregonines, like most other fish species (Guthrie and Muntz 1993), are particulate feeders depending on vision to catch their prey (Janssen 1978; Dabrowski and Jewson 1984), and light intensity is considered to be an important factor controlling their feeding activity (Aksnes and Giske 1993). Fish feeding under sufficient light conditions benefit from better visibility of prey as well as higher reactive volume (Townsend and Risebrow 1982; Ryer et al. 2002; Pekcan-Hekim and Horppila 2007). However, most of our understanding of fish feeding ecology is based on studies conducted under moderate to high light levels, and foraging models for planktivores assume negligible feeding rates at extremely low light intensity (Giske and Salvanes 1995; Hölker and Breckling 2001). Moreover, most studies employ a light spectrum commonly used for room illumination, although the spectrum of the light changes considerably with increasing water depth towards predominantly blue wavelengths (Jerlov 1976; Gerking 1994). It is known that the spectral composition influences the consumption rate of zooplanktivorous fish, because reaction distance is reduced as the wavelength of light decreases from red to blue (Dabrowski and Jewson 1984; Henderson and Northcote 1985).

In the deep and oligotrophic Lake Stechlin (Germany), vendace (*Coregonus albula*) is the dominant pelagic fish species living in sympatry with the endemic dwarf-sized Fontanae cisco (*Coregonus fontanae*; Schulz and Freyhof 2003). The species are easily distinguished by spawning time, with vendace spawning in winter and Fontanae cisco spawning from spring to summer. The species have been described distinct by morphological characteristics (Schulz and Freyhof 2003), and it has been suggested from mtDNA and microsatellite analyses that this species pair evolved via sympatric speciation (Schulz et al. 2006). Both species are planktivores inhabiting the pelagic habitat where they perform diel vertical migrations by ascending from their deepwater daytime habitat into shallower water layers during nighttime (Helland et al. 2007; Mehner et al. 2007). No species-specific difference in diet composition has been observed (Schulz et al. 2003; Helland et al. 2008), and this contrasts the commonly observed divergence along the limnetic–benthic axes found in many other sympatric species in temperate freshwater systems (Lu and Bernatchez 1999; McKinnon and Rundle 2002; Knudsen et al. 2006). Habitat segregation between the Lake Stechlin coregonines has only been observed with respect to vertical distribution within the pelagic habitat (Helland et al. 2007) with Fontane cisco occurring on average in deeper waters than vendace. Zooplankton density, besides light intensity and temperature, also declines continuously with depth in Lake Stechlin (Mehner et al. 2005). Moreover, owing to the weak ecological segregation, high exploitative competition between the species can be assumed. Hence, segregating strategies for reducing interspecific competition were expected according to the competition exclusion principle (see Pianka 2000).

Accordingly, we hypothesized that the species would show a divergence in their efficiency to consume zooplankton. Such a divergence would suggest heritable trait differences, since the fish were hatched and raised under the same laboratory conditions. We investigated the influence of light intensity and prey density on the functional response curves of both species. We hypothesized that Fontane cisco is more efficient at those low light levels and low prey densities, which are typical for the deepwater areas, whereas vendace should be superior at the brighter light and higher prey densities found in lower water depths, where this species is commonly found.

Materials and methods

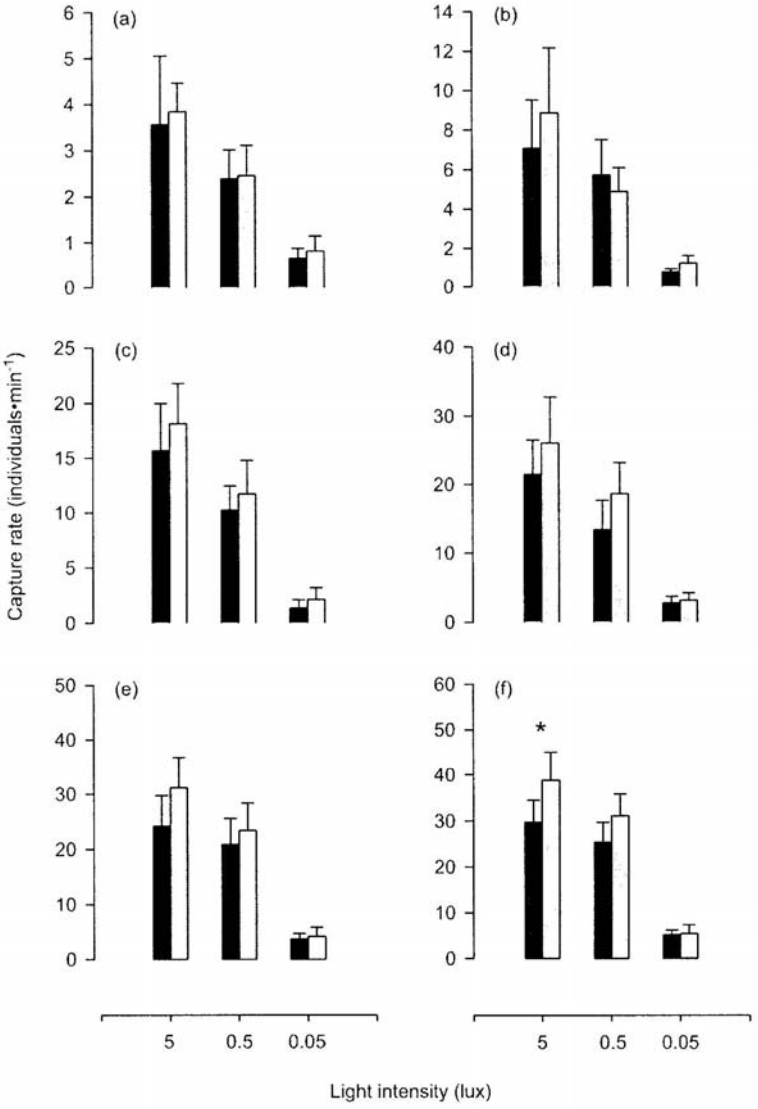
Study species

Fontane cisco and vendace were caught by gillnetting (15 mm mesh size) during their respective spawning times in Lake Stechlin (53°10′N, 13°02′E), Germany. Ripe adults were striped and propagated artificially, and both species were raised under identical laboratory conditions. Larvae were hatched in glass aquaria and subsequently raised in circular basins (80 L). They were fed with brine shrimp, rotifers, and commercially available dry food for fish larvae. Juveniles were maintained in 500 L basins and fed with salmon dry food and *Daphnia magna* to habituate to this prey type. The fish were held at the experimental temperature of 8 °C for at least 2 weeks before experiments started. The prey species *Daphnia magna* was held in circular basins fed with *Desmodesmus subspicatus* algae.

Experimental design

Functional response tests were carried out in six 110 L cubic glass aquaria (48 cm × 48 cm × 48 cm) that were placed in a climatic chamber. The aquaria were filled with 80 L aerated fresh water up to a fill level of 35 cm. The climatic chamber was darkened with light-impermeable curtains inside and outside the entrance. Another row of dark curtains was installed as blinds in front of the aquaria with small peepholes for observation of the feeding activity. Small plastic reservoirs above each of the aquaria served as holding units for *Daphnia* for the feeding experiments. The outlets of these reservoirs were equipped with a valve that opened a tube for the introduction of the *Daphnia* into the aquaria approximately 10 cm below the water surface, which resulted in a homogeneous distribution of the *Daphnia* throughout the entire arena within a short time period. The valves were connected via a stick to a hand gear outside the blinds to introduce the prey without disturbing the fish. Luminaries equipped with 60 W halogen lamps were installed above each of the aquaria. The lamps were connected to a six-channel digital–analogue dimmer (Eurolight LD6230, Behringer, Germany) to allow fine-tuning of the light intensity at very low levels. The intensity of the light was set on a percent scale according to an exponential transmission characteristic of the dimmer. The color temperature of the flashlights (2.800 kelvin (K)) was converted to approximately 20.000 K using tungsten conversion filters (E-Colour+, Rosco, UK). It was adjusted to this value by

Fig. 1. Capture rates (mean and standard error) of Fontane cisco (*Coregonus fontanae*, black bars) and vendace (*Coregonus albula*, grey bars) at 5, 0.5, and 0.05 lx for all prey densities (individuals·L^{-1}): (*a*) 0.25; (*b*) 0.5; (*c*) 1; (*d*) 2; (*e*) 4; (*f*) 8. Differences between light intensities were significant between 0.5 and 0.05 lx at all prey densities in both species, but only partly significant between 5 and 0.5 lx. The only significant difference between species was found at 8 individuals·L^{-1} at 5 lx, as indicated by an asterisk.



placing two conversion filters (Double and Quarter C.T. Blue) on top of each other in front of the lamps behind a framed glass. Color temperature varied from 17.000 to 23.000 K depending on the light intensity used for the respective experiment. The conversion was done to simulate the blue spectrum of the natural habitat, since these fish usually feed in depths of more than 15 m during crepuscular to dark light periods and more than 35 m during daytime according to average year-round population depths (Helland et al. 2007; Mehner et al. 2007). Color temperature was measured with a color analyzer (TF5, Thoma, Germany). Light intensity was measured at mean depth in the aquaria using a light detector (SUD033/Y/W) connected to a research radiometer (IL 1700, International Light Technologies, Massachusetts, USA). The light detector had a measurement range of 400–700 nm and a dynamic range of 8×10^{-4} to 8×10^{5} lx.

Infrared lamps (880 nm, 60 W, WRL-I/LED06, Videor Technical, Germany) were installed above each of the aquaria to allow the observation of feeding activity at very low

Table 1. *P* values of the analysis of variance (ANOVA) Tukey's test for differences between light intensities at all prey densities.

	Coregonus albula		*Coregonus fontanae*	
Prey·L^{-1}	5 vs. 0.5 lx	0.5 vs. 0.05 lx	5 vs. 0.5 lx	0.5 vs. 0.05 lx
0.25	0.056	0.022	0.141	0.013
0.5	0.022	0.036	0.842	0.004
1	0.014	<0.001	0.026	<0.001
2	0.110	<0.001	0.063	0.008
4	0.044	<0.001	0.751	<0.001
8	0.071	<0.001	0.645	<0.001

Note: In both species, differences between 5 and 0.5 lx were partly significant, whereas differences between 0.5 and 0.05 lx were entirely significant.

Table 2. *P* values of the analysis of variance (ANOVA) Tukey's test for differences between species at all prey densities and light intensities.

Prey·L^{-1}	5 lx	0.5 lx	0.05 lx
0.25	0.990	0.999	0.999
0.5	0.640	0.978	0.999
1	0.696	0.949	0.997
2	0.570	0.409	0.999
4	0.092	0.908	0.999
8	0.016	0.404	0.999

Note: The only significant difference in consumption rate was found at the highest light intensity (5 lx) and prey density (8 individuals·L^{-1}) combination.

light intensities. This was done using night-vision glasses (D-2MV PRO. Hupra, Germany), which allowed the observation of the fish as well as the *Daphnia* during the experiments. Hence, it was possible to track an individual fish and its consumption of *Daphnia* through the peepholes of the aquaria without disturbing it.

Experimental protocol

Functional response experiments were performed at a water temperature of 8 °C at four different light intensities (0.005 ± 0.001, 0.05 ± 0.005, 0.5 ± 0.02, and 5 ± 0.01 lx). Six prey densities of 0.25, 0.5, 1, 2, 4, and 8 *Daphnia*·L^{-1} were used at each of the four light intensities. These prey densities were the mean densities over a feeding trail where each fish was allowed to eat six *Daphnia*. Accordingly, the total number of *Daphnia* introduced into the 80 L aquaria was 23, 43, 83, 163, 323, and 643 individuals for the six densities, respectively. Since the experiments were terminated after six *Daphnia* were captured, prey depletion was 26.1%, 14.0%, 7.2%, 3.7%, 1.9%, and 0.9% for the increasing prey densities, respectively. We therefore assume that the modest ratios of prey depletion did not substantially bias the results.

One fish was placed in each of the six aquaria, which thereby served as replicates for the specific light intensity and prey density combinations. The subsequent acclimation to experimental conditions was allowed overnight at the light intensity of the upcoming experiment. Individuals were randomly selected from a 500 L tank holding all potential experimental fish. Specimens used for this study were all juveniles and measured 6–11 cm (mean ± standard deviation, SD: 8.3 ± 1.3 cm) and 6–13 cm (mean ± SD: 9.7 ± 1.7 cm) total length for Fontane cisco and vendace, respectively. On the next day, prior to the experiments, *Daphnia* of 1.5–3 mm in size were sieved out of their holding tanks, counted, and poured into the small plastic reservoirs above the aquaria. After another 3 h, trails started with the introduction of the prey into one aquarium at a time. The time from the beginning of the feeding activity until the fish had eaten six *Daphnia* was measured using a stopwatch with an intermediate memory function. Typically, at the beginning of an experiment the fish stayed near the bottom. After perceiving the first *Daphnia*, the fish started to swim more actively, thereby searching for prey. At that time the *Daphnia* were already quite evenly distributed throughout the arena. The fish usually used the entire arena to forage continuously. After all six trails were counted, the fish were removed from the aquaria. Subsequently, the water was sieved to remove the remaining *Daphnia* and to prepare for the next experiment.

Data analysis

Three types of functional responses are distinguished: (*i*) the linear type I response with consumption rate increasing linearly with prey density until a satiation value is reached, with $N = \alpha P$, where N is the consumption rate (individuals·L^{-1}), P is prey density (individuals·L^{-1}), and α is the slope of the consumption rate; (*ii*) the cyrtoid type II response with consumption rate increasing with prey density at a decreasing rate until satiation is reached, with $N = \beta_1 P(\beta_2 + P)^{-1}$, where β_1 is the maximum consumption rate, and β_2 is the prey density at which the consumption rate reaches half of its maximum (Holling 1959; Arditi and Ginzburg 1989); and (*iii*) the sigmoid type III response with consumption rate accelerating first with increasing prey density and thereafter decelerating towards satiation, with $N = \beta_1 P^2(\beta_2 + P^2)^{-1}$. We used linear and nonlinear regression analyses to fit these functional response curves.

A method based on information criterion was used to determine the regression model for the functional response that best described the experimental data. We used the Schwarz–Bayesian information criterion (S–BIC, Schwarz 1978). It is a restrictive criterion on increasing parameter numbers: S–BIC = $k\ln(n) + n\ln(\text{RSS}\cdot n^{-1})$, where k is the given number of parameters, n is the number of observations, and RSS is the residual sum of squares. The model associated with smallest value of S–BIC is the most appropriate.

Statistics

Functional response data were compared for significant differences using the following tests based on a significance level of $P < 0.05$. Analysis of variance (ANOVA. Tukey's test) was used to compare the mean values between species and light intensities at a particular prey density. To evaluate the influence of fish length, an analysis of covariance (ANCOVA) was performed. Further, we linearized the functional response data by taking the $\log_{10}$ of

Table 3. Statistical results and selection criterion for the best-fit models of the functional response according to types I, II, and III curves for both species at all three light intensities.

	Coregonus albula			*Coregonus fontanae*		
Model	Type I	Type II	Type III	Type I	Type II	Type III
5 lx						
k	1	2	2	1	2	2
n	33	33	33	33	33	33
df	32	31	31	32	31	31
RSS	3369.92	786.78	1000.82	3226.95	1062.13	1097.19
S–BIC	156.16	111.65	119.59	154.73	121.55	122.63
0.5 lx						
k	1	2	2	1	2	2
n	31	31	31	33	33	33
df	30	29	29	32	31	31
RSS	1732.65	646.66	728.51	1333.18	347.62	432.05
S–BIC	128.16	101.04	104.74	125.56	84.70	91.87
0.05 lx						
k	1	2	2	1	2	2
n	30	30	30	29	29	29
df	29	28	28	28	27	27
RSS	70.20	35.85	44.53	37.67	27.10	32.38
S–BIC	28.90	12.15	18.65	10.95	4.76	9.93

Note: The model associated with the lowest values in S–BIC was selected as the most appropriate. Abbreviations are as follows: *k*, number of parameters used; *n*, total number; df, degrees of freedom; RSS, residual sum of squares; S–BIC, Schwarz–Bayesian information criterion

prey density and compared the capture rate with prey density relationship between the species by ANCOVA. ANOVA and ANCOVA tests were performed using SPSS 14.0 (SPSS Inc., Chicago, Illinois). The estimated mean values for β_1 and β_2 were tested for significance using a *t* test.

Results

Capture rates in both species generally increased with prey density. Mean capture rates for both species at all prey densities and light intensities are shown (Fig. 1). No feeding activity was observed at 0.005 lx. In both species, differences between light intensities were all significant when 0.5 and 0.05 lx were compared, but only partly significant when 5 and 0.5 lx were compared (ANOVA, Tukey's test, Table 1). Significant differences between the species were found at the highest light intensity and prey density combination (5 lx and 8 individuals·L^{-1}). All other 17 interspecies comparisons were not significant. The differences between the species generally tend to increase with increasing light intensity and prey density (ANOVA, Tukey's test, Table 2). Fish size, measured as total body length, did not significantly affect capture rates (ANCOVA, $P > 0.1$ at all prey densities).

According to the S–BIC, the functional response type II gave the best fits to the data sets at all light intensities (5, 0.5, and 0.05 lx) in both species. However, the type III function gave comparably good fits in some cases (Table 3). The increase in capture rate with prey density at all light intensities is shown (Fig. 2). Capture success was depressed with decreasing light intensity in both species. After log-transforming prey density, ANCOVA showed a significant difference between the species at 5 lx ($P = 0.003$), but no significant difference at 0.5 lx ($P = 0.051$) and 0.05 lx ($P = 0.129$). The estimated parameters of the functional response type II fits are presented (Fig. 3, Table 4). According to the *t* test, maximum feeding rates were significantly higher in vendace compared with Fontane cisco at 5 lx (df = 10, $P = 2.839$), but there was no significant difference between species at 0.5 lx (df = 10, $P = 1.568$) and 0.05 lx (df = 10, $P = 0.563$). Differences between light intensities were not significant between 5 and 0.5 lx, but were significant between 0.5 and 0.05 lx in both species. Prey densities of half maximum feeding rate were not significantly different between species at all light intensities (5 lx: df = 10, $P = 0.699$; 0.5 lx: df = 10, $P = 0.284$; 0.05 lx: df = 8, $P = 1.151$).

Discussion

The consumption rate of Fontane cisco and vendace feeding on *Daphnia magna* increased with increasing prey density at a decreasing rate until satiation was reached. It thus followed a type II functional response. This type of response has been reported for other coregonines, for instance bloater (*Coregonus hoyi*; Miller et al. 1992), and was found in a recent study on a Swedish vendace population (U. Beier, Swedish Board of Fisheries, Drottningholm, Sweden, unpublished data). Some studies have reported a linear type I or a sigmoid type III functional response for planktivores (e.g., Winkler and Orellana 1992; Ryer et al. 2002), and a shift from type II to another response at low light intensity has also been described (Townsend and Risebrow 1982;

Fig. 2. Capture rates as a function of prey density of Fontane cisco (*Coregonus fontanae*, black triangles and black lines) and vendace (*Coregonus albula*, grey triangles and grey lines) at (*a*) 5 lx, (*b*) 0.5 lx, and (*c*) 0.05 lx. Shown are all individual data and a curve fitted according to a Holling type II functional response, which was determined as the best-fit model. Capture rate increases with increasing prey density at all light intensities in both species. Fontane cisco showed a significantly lower functional response compared with vendace at 5 lx ($P = 0.003$), but not at 0.5 lx ($P = 0.051$) and 0.05 lx ($P = 0.129$).

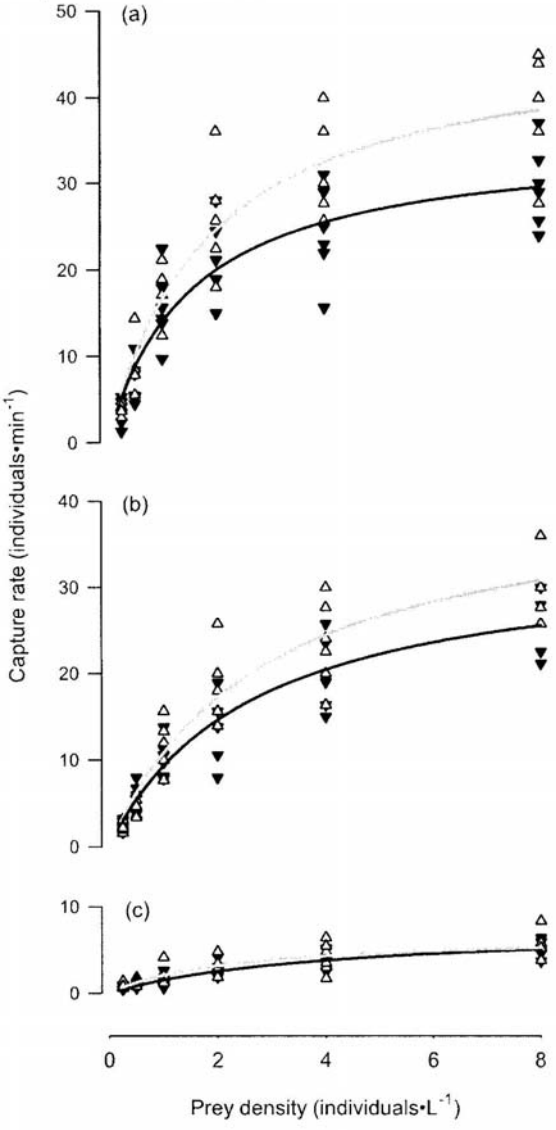
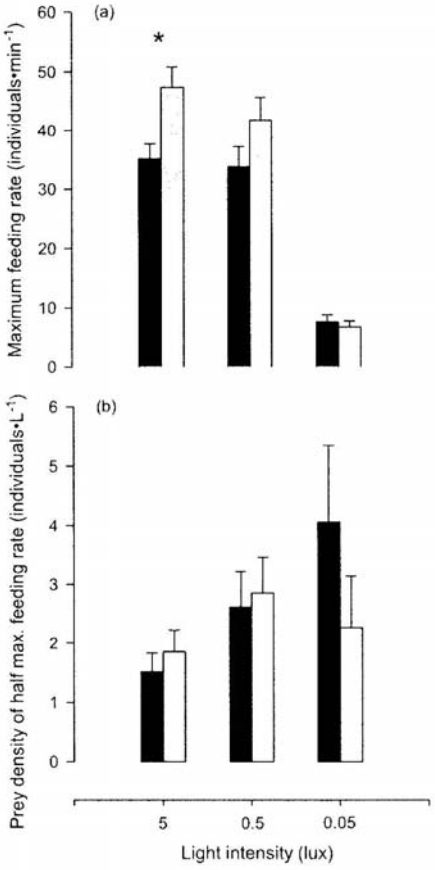


Fig. 3. (*a*) Maximum feeding rates and (*b*) prey densities of half maximum feeding rates of Fontane cisco (*Coregonus fontanae*, black bars) and vendace (*Coregonus albula*, grey bars) at 5, 0.5, and 0.05 lx. Shown are parameter estimates of the functional response type II fits and their standard errors. Maximum feeding rate significantly increases between 0.05 and 0.5 lx, whereas differences in prey density of half maximum feeding rate are not significant. Interspecies differences are significant only for the maximum feeding rate at 5 lx, as indicated by an asterisk.



Koski and Johnson 2002). However, the type II function is most frequently observed in planktivorous fishes (Jeschke et al. 2004). It has to be mentioned that different approaches for analyzing functional response data have been proposed (see Juliano 2001).

The two planktivorous coregonines of Lake Stechlin showed a marginal divergence in their feeding behaviour. Significant differences in functional response were only found at the highest light intensity and when associated with high food density. At all other light and food conditions investigated in this study, the species showed no difference in functional response. The combination of high food abundance and relatively high light intensity seems to represent a rather extreme condition. We re-examined field data based on zooplankton samplings from Lake Stechlin (Helland et al. 2007) and computed a mean zooplankton density for the most relevant prey species of the coregonines, including daphnids, small cladocerans, and copepods

Table 4. Estimates and standard errors for parameters of the type II functional responses for both species at all three light intensities.

	Coregonus albula			*Coregonus fontanae*			Comparison	
	n	Estimate	SE	*n*	Estimate	SE	df	P
5 lx								
β_1	6	47.43	3.47	6	35.16	2.58	10	2.839
β_2	6	1.85	0.37	6	1.51	0.32	10	0.699
0.5 lx								
β_1	6	41.85	3.82	6	33.84	3.38	10	1.568
β_2	6	2.86	0.60	6	2.62	0.60	10	0.284
0.05 lx								
β_1	5	6.77	1.05	5	7.66	1.18	8	0.563
β_2	5	2.27	0.87	5	4.06	1.29	8	1.151

Note: The only significant difference between the species was found for β_1 at 5 lx. β_1, maximum consumption rate (individuals·min^{-1}); β_2, prey density of half maximum consumption rate; *n*, total number; SE, standard error; df, degrees of freedom.

of about 2.5 individuals·L^{-1} below 10 m depth. High food abundances of 8 individuals·L^{-1} or more in these depths are found only for some weeks during summer. Further, hydroacoustic surveys in the lake suggest that the fish feed in depths where light intensity is seldom at a level of 5 lx or above. The species stay below 35 m depth during day and migrate to lower depth during crepuscular light and at nighttime, so that foraging is generally associated with lower light intensities (S. Busch and T. Mehner, unpublished data). Thus, because of the migration pattern, a combination of high light intensity and high food density can be assumed to be very unlikely (i.e., for the most relevant environmental conditions found in situ, the species do not differ considerably with respect to their feeding efficiency). However, vendace feeding was slightly more efficient than that of the deeper living Fontane cisco at the combination of the highest light intensity and the highest food level, suggesting a beginning or ongoing segregation in their functional response along the light gradient.

Light intensity had a major influence on the feeding behaviour in both species, with declining efficiency down to a threshold between 0.05 and 0.005 lx. Generally, it is in accordance with many other studies on fish feeding behaviour that capture success is reduced as light intensity decreases. However, ongoing feeding activity of fishes that usually use vision for prey capture has been reported under light conditions approaching darkness in some species (e.g., Janssen 1978; Townsend and Risebrow 1982; Pekcan-Hekim and Horppila 2007). Hence, fishes might rely on nonvisual senses, for example the mechanosensory lateral line system, to locate and attack prey under light conditions where vision is ineffective (Janssen and Corcoran 1993; Hölker and Thiel 1998; Liang et al. 1998). The lack of feeding activity at the lowest light intensity (0.005 lx) investigated in this study suggests that the coregonines are unable to feed when light intensity approaches zero. However, we have to note that an effect of light spectrum on foraging behaviour cannot be completely ruled out. Hence, the described influence of light intensity on the feeding efficiency may possibly be confounded by an effect of color spectrum. This is contrasted by the fact that the change of color temperature during the experiments was associated with relatively minor changes in transmitted wavelength, since the color filter we used had a transmission characteristic of 50%–65% at 400–500 nm wavelengths but only 5%–10% in the 550–700 nm range, leaving mainly the blue and violet wavelengths just like clear water in depths of 15 m and more. Moreover, feeding activity of coregonines is generally discussed to be strongly influenced by light intensity and its diel and seasonal patterns. Dabrowski and Jewson (1984) showed that the feeding activity of Irish pollan larvae (*Coregonus pollan*) was strongly determined by light intensity and Gjelland et al. (2004) reported that feeding activity of pelagic common whitefish (*Coregonus lavaretus*) and vendace was more strongly correlated to light intensity than to circadian rhythms.

The diel vertical migration of the Lake Stechlin coregonines may be explained by the bioenergetics efficiency, the feeding opportunity, or the predator avoidance hypotheses (Scheuerell and Schindler 2003). If the latter applies, this would emphasize the importance of feeding at low light intensities to avoid predation, since yellow perch (*Perca flavescens*) as the most abundant predator species in the lake usually forage in shallow water at moderate to high light levels. However, hydroacoustic surveys from Lake Stechlin suggest that the seasonal variation of depth distribution of both species is more strongly correlated to temperature than to water transparency, and thus the migration patterns of these coregonines are only weakly related to the light regime and the occurrence of the most important food resources and potential predatory fish (Mehner et al. 2007). Hence, there is only little support for the predator avoidance as well as the feeding opportunity hypotheses, favoring the bioenergetics efficiency hypothesis to explain diel vertical migrations and putting forward temperature as the ultimate abiotic factor driving species' vertical orientation.

Our results clearly demonstrate a significant influence of food density and light intensity on capture rates in both species. Since they show an ecological segregation with respect to their vertical distribution within the pelagic habitat (Helland et al. 2007), where light intensity and food density decline continuously with depth (Mehner et al. 2005), these gradients induce severe species-specific consequences with respect to energy budgeting. The difference in average population depth imposes energetic disadvantages on the deeper-living Fontane cisco, because this species encounters lower food abundances under less favorable light conditions compared with sympatric vendace without showing any compensatory mechanism via enhanced feeding efficiency. This conclusion is supported by growth analysis (Schulz and Freyhof 2003) showing that individuals of Fontane cisco have a lower growth rate compared with vendace. That raises the question how this species is able to sustain a life-supporting energy budget under such unfavorable conditions and high exploitative competition with vendace.

The cooler temperatures at greater depths reduce the metabolic requirements considerably. An average temperature difference of only 1 °C means 7%–10% less metabolic costs for Fontane cisco. Furthermore, it appears that this species has adapted to the unfavorable conditions by reducing its standard metabolic rate. We found substantially lower standard costs in Fontane cisco compared with vendace over the

whole range of environmental temperatures (Ohlberger et al. 2008). Although the relative amount of energy for metabolism of dwarf fish could be higher (Trudel et al. 2001), dwarfism per se might also be an adaptive strategy to living in deeper waters, instead of being its consequence, since smaller body size means on average less total energy expenditure for maintenance. Svärdson (1979) argued that in a situation of reduced food ration due to competition between pelagically living sympatric forms, the "dominated", inferior population could have a better energy budget if it withdraws to deeper and cooler waters. Further, the lower energy costs of smaller fish inspired Hamrin and Persson (1986) to base their hypothesis about the oscillation cycles in single vendace populations on a principle of competition between age classes. They argued that small vendace experience a competitive advantage compared with larger fish during periods of low food abundance because of their lower metabolic requirements.

In summary, in contrast with our hypothesis, the ecological segregation between the co-occurring species is not facilitated by differences in feeding efficiency, since their functional response curves barely differed within the natural range of environmental conditions. Instead, together with a study on the species' feeding preference, our data suggest that these coregonines are functionally similar, coexisting planktivores that only use slightly different feeding niches within their pelagic environment. We conclude that species' coexistence may be facilitated by alternative energetic strategies and recommend this as a promising field for future research.

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Paper II

II

Effects of temperature, swimming speed and body mass on standard and active metabolic rate in vendace (*Coregonus albula*)

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ORIGINAL PAPER

Effects of temperature, swimming speed and body mass on standard and active metabolic rate in vendace (*Coregonus albula*)

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Abstract This study gives an integrated analysis of the effects of temperature, swimming speed and body mass on standard metabolism and aerobic swimming performance in vendace (*Coregonus albula* (L.)). The metabolic rate was investigated at 4, 8 and 15°C using one flow-through respirometer and two intermittent-flow swim tunnels. We found that the standard metabolic rate (SMR), which increased significantly with temperature, accounted for up to 2/3 of the total swimming costs at optimum speed (U_{opt}), although mean U_{opt} was high, ranging from 2.0 to 2.8 body lengths per second. Net swimming costs increased with swimming speed, but showed no clear trend with temperature. The influence of body mass on the metabolic rate varied with temperature and activity level resulting in scaling exponents (b) of 0.71–0.94. A multivariate regression analysis was performed to integrate the effects of temperature, speed and mass ($AMR = 0.82 M^{0.93} \exp(0.07T) + 0.43M^{0.93}U^{2.03}$). The regression analysis showed that temperature affects standard but not net active metabolic costs in this species. Further, we conclude that a low speed exponent, high optimum speeds and high ratios of standard to activity costs suggest a remarkably efficient swimming performance in vendace.

Keywords *Coregonus spp.* · Energetic costs · Metabolic rate · Swimming performance · Temperature

Abbreviations

AMR	Active metabolic rate (J s^{-1})
BL	Body length (m)
COT	Cost of transport when swimming at U_{opt} (J m^{-1})
M	Fish mass (kg)
net	Refers to metabolic rate excluding SMR (–)
SMR	Standard metabolic rate (J s^{-1})
T	Temperature (°C)
U	Swimming speed (m s^{-1})
U_{opt}	U associated with minimum costs (m s^{-1})

Introduction

The capacity for movement in fish is directly related to food capture, habitat shift and reproduction, and it is considered a main trait determining ecological fitness (Videler 1993; Plaut 2001). The swimming performance of fishes responds to a variety of environmental factors such as diet, photoperiod, season, oxygen tension and temperature (Fry 1971; Webb 1975). Multi-factor analyses of the energetic costs during swimming are fundamental to understand the effects of extrinsic as well as intrinsic factors on the swimming capacity. It has been suggested that fish mass and swimming speed are the most important and significant factors influencing the energy turnover during swimming (Boisclair and Tang 1993), and temperature is known to be

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the most important environmental factor when the standard metabolism is considered.

The influence of temperature on the metabolic rate is of particular relevance for freshwater fishes in the temperate zone that are subjected to severe seasonal temperature changes. The temperature dependence of the standard or routine metabolic rate in animals is generally described by an exponential relationship. The corresponding temperature coefficient rages from 0.05 to 0.10 in vertebrates (White et al. 2006). The argument whether this coefficient is universal, like suggested by Gillooly et al. (2001), continues up to date (Clarke 2006; Gillooly et al. 2006). On the other hand, the influence of temperature on the active metabolic rate, especially the net activity costs, remains unclear. Different metabolic rates or swimming behaviours might be affected differently by temperature due to their different physiological bases. Various studies indicated that environmental temperature affects the swimming performance in fish (Tang et al. 2000; Lee et al. 2003; Day and Butler 2005; Claireaux et al. 2006), but nevertheless, its impact remains poorly understood due to the diversity of interacting physiological, biochemical and behavioural processes that are involved (Taylor et al. 1997). Furthermore, the few studies that have investigated the effect of thermal acclimatisation upon net activity costs described a lack of thermal effect (Beamish 1990; Claireaux et al. 2006). However, there are some examples in the literature where net swimming cost was shown to be influenced by water temperature (Johnston and Temple 2002; Dickson et al. 2002).

The energy expenditure during locomotion is further influenced by the swimming velocity of a fish. With increasing velocity, the energy expenditure for propulsion becomes more important with respect to the total energetic costs while the influence of other factors diminishes. Webb (1975) suggested that swimming cost is a power function of speed with an exponent of 2.5 under laminar flow conditions, but other authors computed distinctly lower exponents (e.g. Beamish 1978). A comparison of coefficients reported in the fish physiology literature, either determined by single- or multi-factor analyses, is difficult due to the variety of regression methods used, which include linear, semi-logarithmic or logarithmic relationships (Webb 1975; Beamish 1978; Videler and Nolet 1990; Wieser 1991; Boisclair and Tang 1993; Ohlberger et al. 2005).

The other important intrinsic parameter affecting the energy consumption of an animal is its body mass. The metabolic rate, like many other physiological processes, is related to body mass by an allometric relationship (Winberg 1961; Schmidt-Nielsen 1972). It has been assumed that the scaling exponent of this relationship is 0.75 for all animals and plants, derived from the physics of distribution networks (West et al. 1997; Brown et al. 2004). However, broad literature reviews revealed significant allometric exponent heterogeneity between various animal groups (White et al. 2006; Glazier 2005). Moreover, ectotherms seem to have higher exponents compared to endotherms (McNab 2002) and values found in the fish physiology literature vary considerably.

An integrated analysis of the effects of temperature, swimming speed and body mass on standard and net activity costs in vendace (*Coregonus albula* (L.)) is provided in this study by means of a multivariate non-linear regression analysis. Moreover, the swimming speed associated with minimum costs per unit distance (U_{opt}) and the energy expenditure when swimming at this speed (COT), representing two ecologically important measures of the swimming performance, were computed for this species.

Vendace is a typically lacustrine coregonid that mainly occurs in northern and Baltic Europe. It is the dominant pelagic fish species in the dimictic, oligotrophic Lake Stechlin, Germany (Mehner and Schulz 2002). Vendace prefers high concentrations of dissolved oxygen and low water temperatures (see Helland et al. 2007). Mean environmental temperature at the population depth for vendace in Lake Stechlin ranges year-round from 3.3 to 7.3°C, but the fish encounter higher water temperatures during food capture since they perform diel vertical migration to lower water depths (Helland et al. 2007). It can be assumed that vendace feature an efficient swimming performance due to its streamlined body form. In this study, we present the first ever published data of active metabolic costs in this species.

Materials and methods

Fish hatching and maintenance

Autumn/winter spawning vendace (*Coregonus albula*) were caught in late December by gill netting (15 mm mesh size) from Lake Stechlin (53°10′N, 13°02′E), Germany. Ripe adults were striped and propagated artificially. Larvae were hatched at 4°C, subsequently raised in circular basins (80 l) and fed with brine shrimp (*Artemia salina*), rotifers (*Brachionus* spp.) and commercially available dry food. At a size of approximately 10 cm, fish were transferred to 500–1,000 l basins and fed with salmon dry food only. The study was conducted in consideration of the seasonal temperatures in the lake, i.e. experiments were carried out in winter, autumn or spring and summer for the 4, 8 and 15°C, respectively. The fish were held at the respective experimental temperatures for at least one week before experiments started. Feeding was interrupted 48 h prior to the experiments to avoid elevated oxygen consumption

rates due to specific dynamic action. Vendace used in this study had a length of 10–22 cm and a body mass of 10–75 g.

Experimental design

The experiments were conducted with three different respirometers. One had a simple flow-through design, where fish were placed in small measuring chambers with a minimized water flow to determine the standard metabolic rate. The other two were Brett-type tunnel respirometers (Brett 1964) with an automated and computerised intermittent-flow system to measure the active metabolic rates during swimming. All respirometers were placed in a climatic chamber and equipped with a chilling unit for fine-tuning of the temperature.

SMR measurements

The standard oxygen consumption of the fish was measured with a flow-through respirometer (Ludolph, Bremerhaven, Germany). It consisted of six Plexiglas chambers surrounding a central cone with the oxygen sensor (type 1000-200) connected to an oxymeter (M200, both Eschweiler, Kiel, Germany). Each measuring unit (680 ml) was supplied by aerated fresh water from the reservoir tank surrounding the six chambers. Oxygen rates were measured alternately by switching the direction of the central cone. A tubing pump (Ismatec, Wertheim-Mondfeld, Germany) generated a water flow through the chamber into the central cone where the oxygen electrode was installed to measure the partial oxygen pressure of the outflow. Flow velocity was 53 ml min^{-1} avoiding a potential drop of the oxygen concentration beneath 80% saturation. Oxygen saturation of the inflow was set to 100% as the reservoir was aerated and circulated though an UV sterilizer constantly.

Individual fish were introduced into fife of the respirometer chambers at least 24 h prior to measurements to allow adaptation to the experimental conditions. The sixth chamber was used to measure a blank value, which accounted for up to 15% of the oxygen depletion. Oxygen consumption of a single fish was recorded continuously for a period of approximately 1 h with a sampling interval of 0.5 s. Data were analysed using Chart v. 4.0.1 (PowerLab, ADInstruments, Oxfordshire, UK). Oxygen consumption rates (MO_2, mgO_2 h^{-1}) were calculated using the equation:

$$MO_2 = \Delta PO_2 \beta M V_{fl}, \quad (1)$$

where ΔPO_2 is the difference in oxygen content between in- and outflow (Pa), β is the oxygen capacity of the water (mol l^{-1} Pa^{-1}), M is the molar mass of the oxygen (mg mol^{-1}) and V_{fl} is the flow rate though the chamber (l h^{-1}). Only data from the lowest 10% of the calculated oxygen consumption rates were used for the SMR analysis.

Moreover, data from fish that showed permanent spontaneous activity even within the small chambers used for this analysis were excluded from further SMR calculations. The scaling relationship of the SMR data of all individuals ($N = 56$) was described by the commonly used allometric function:

$$SMR = aM^b, \quad (2)$$

where SMR is the metabolic rate at complete rest, M is body mass and b is the mass exponent. The regression of the direct SMR data at 15°C was compared to the regression of the extrapolated SMR values from the swim tunnel experiments at this temperature. Since the extrapolation to zero speed proved to be a reliable method for estimating the SMR, further analyses were conducted with the estimates from the swim tunnel measurements.

AMR measurements

Active metabolic rates were determined using two different automated intermittent-flow systems, one for smaller fish of 10–25 g and the other for fish larger than 25 g body mass. Due to the relatively small fish sizes used in both respirometers, a correction for solid blocking effects was not necessary in either of the swim tunnels, because fish cross-sectional area did never exceed 10% of the area of the whole swimming chamber (Bell and Terhune 1970). Only relatively small fish were used for the experiments since larger fish were not able to turn easily within the swimming section and hence got stuck at the grid at the end of the section when occasionally attempting to turn at higher speeds.

Large swim tunnel

The design of the respirometer and its data analysis is described in detail in Ohlberger et al. (2005). The swim tunnel for the larger fish was a modified Brett-type tunnel respirometer designed after Hölker (2003). It consisted of a measuring recirculation system (25 l) with a swimming section of 15 cm in diameter and 40 cm in length. A black screen darkening the first part of the swimming section motivated the fish to swim in an upstream position. Water flow was driven by a paddle-wheel pump (BN100-65-125, Jesco, Wedemark, Germany), which was controlled by a frequency changer (NORDAC vector mc, Getriebebau Nord, Bargteheide, Germany). A flow transmitter (+GF+Signet 8550-1, Signet Scientific Company, El Monte, CA, USA) was installed on the pressure side for sensitive velocity adjustments. Flow velocity was calibrated using a

field version of the three-dimensional acoustic Doppler velocity meter (ADV, Nortek AS, Norway). Oxygen concentration was measured with a fixed TriOxmatic 701 oxygen sensor (WTW, Weilheim, Germany) coupled to an oximeter (WTW Oxi 171) that allowed automated measuring and flushing periods via a magnet valve. The respirometer was temperature-controlled to the desired value ±0.2°C. The oxygen content of the water decreased during the measuring phase until a lower threshold was reached. The ventilation connection was opened and aerated fresh water from an external ventilation system (125 l) entered the measuring circuit until the upper threshold was re-established. The upper and lower thresholds were fixed depending on fish mass and swimming speed to have a constant drop of approximately 5% in oxygen saturation during each measuring phase. Minimum lower threshold was 80% oxygen saturation. Oximeter output, including oxygen saturation, temperature and ventilation status, was recorded every 6 s by a computer.

Small swim tunnel

The smaller swim tunnel for intermittent-flow respirometry was a Swim5 acquired from Loligo (Loligo Systems ApS, Hobro, Denmark). The respirometer consisted of a recirculation loop (5 l) with flow generated by a propeller and an ambient tank (20 l) for fresh water supply. Another reservoir with cooled fresh water (60 l) was installed beside the respirometer for a sensitive temperature control of the system. The recirculation loop consisted of deflectors and a honeycomb serving as flow rectifier on the upstream side of the swimming section (10 × 10 × 30 cm). A black screen darkening the first part of the swimming section motivated the fish to swim in an upstream position. The propeller was connected to the motor outside of the respirometer via a submersed coupling. A submersible flush pump in the ambient tank was used for fresh water supply of the measuring section. The oxygen content in the swimming chamber was measured by a dissolved oxygen probe (MINI-DO, Loligo Systems ApS). The whole system was run by the LDAQ instrument from Loligo, connected to a computer via USB port. The instrument (LDAQ) recorded oxygen saturation and temperature in the swimming section and controlled the ambient tank pump for automated flushing as well as a pump for the temperature control that delivered cooled water from the reservoir into the ambient tank. The software LoliResp allowed automated measuring, wait and flush periods, a sensitive temperature control and velocity adjustments of the motor. Water velocity was calibrated to the voltage output of the motor using a three-dimensional acoustic Doppler velocity meter (ADV, Nortek AS, Norway). Measuring phases were set at 500–1,500 s depending on fish mass and swimming speed to ensure that the oxygen content in the measuring section never dropped below 80% saturation. Flush period was set to 80 s and wait period to 30 s. Temperature was set to the desired value with a hysteresis of ±0.1°C.

Experimental protocol

Experiments were conducted from May 2005 to September 2006. At each of the three temperatures (4, 8 and 15°C), 14 vendace were used for the swim tunnel experiments. Fish length was measured prior to the tests. Individuals of 10–15 cm were introduced into the small and those of 15–22 cm into the large swim tunnel. To allow adaptation to experimental conditions, a velocity of 0.5 BL s^{-1} (body length per second) was run for at least 24 h. Subsequently, velocities of 0.75, 1.0, 1.5 and 2.0 BL s^{-1} were run for 8–16 h each, day and overnight. After 5 days of swimming trail, fish were removed from the respirometer and weighted immediately. This was done afterwards to reduce handling stress prior to the experiment. Subsequently, a blank value was determined. Microbial respiration accounted for up to 15 and 35% of the total respiration in the small and large respirometer, respectively (for comparison see Dalla Via 1983).

Data analysis

For the small swim tunnel, the LoliResp software delivered the regression values of the acute oxygen consumption in the respirometer during each measuring period ($mgO_2\ kg^{-1}\ h^{-1}$). For the large respirometer the acute oxygen consumption rate of the fish was computed by fitting a linear regression to the decrease in oxygen saturation against time for each phase of closed respirometry. All oxygen consumption values were corrected for bacterial respiration.

To distinguish between adaptation and routine phase of swimming, the procedure after Hermann and Enders (2000) was applied to the 0.5 BL s^{-1} data. Adaptation phases in vendace lasted for up to 12 h. Data from the adaptation phase were excluded from further calculation. The active metabolic rate for every single swimming velocity was computed from the lowest 10% of all values. This was done to determine the minimum active metabolic rate without any spontaneous activity or stress phases the fish might have experienced during the experiment.

A power function was used to describe the AMR to speed relationship (Videler and Nolet 1990; Korsmeyer et al. 2002):

$$\mathrm{AMR} = a + bU^c, \tag{3}$$

where a, b and c are constants and U is swimming speed (BL s^{-1}). Constant a represents the estimated SMR, i.e.

metabolic rate at zero speed, and c is the speed exponent. The estimated SMR values (a) were plotted against body mass for all three temperatures and mass exponents (b) were calculated according to the allometric function (Eq. 2). Further, to evaluate the effect of temperature on the active metabolism, AMR values were corrected for body mass using the mass exponents derived for the relationship between standard rate and body mass. Subtracting the SMR from these values delivered the mass-corrected net active metabolic rate (net AMR).

Differentiation of Eq. 3 with respect to U and zero-setting gives the swimming speed associated with minimum energetic costs per unit distance, which is also referred to as optimum speed (U_{opt}). It is thus the speed where the ratio of the active metabolic rate to swimming speed reaches a minimum (Tucker 1970):

$$U_{opt} = [a/b(c-1)]^{1/c}, \quad (4)$$

The energy expenditure when swimming at U_{opt} is called the cost of transport (Videler and Nolet 1990). It is the minimum energy needed by a fish to swim one unit distance (J m^{-1}):

$$COT = AMR_{opt}/U_{opt}, \quad (5)$$

where AMR_{opt} (J s^{-1}) is active metabolic rate at U_{opt} (m s^{-1}). Subtracting the SMR from the AMR_{opt} value in this equation delivers the net cost of transport (net COT). All data of the oxygen consumption rates were converted into energy units using an oxycaloric value of 14.2 mg O_2^{-1} (Hepher 1988).

Regression analysis and model selection criteria

To evaluate the integrative effects of temperature, body mass and swimming speed on the metabolic rate, a multivariate non-linear regression analysis was performed. The formula to describe the empirical data was designed according to the following considerations. The mass to metabolic rate relationship is generally assumed to have an allometric form and was thus modelled using a power fit (M^b). Temperature is generally described by an exponential relationship to metabolic rate and was hence modelled by an exponential function with a temperature coefficient (e^{dT}). The relationship between swimming speed and metabolic rate was again described by a power fit (U^c), since this method produced better fits than the exponential function when the individual data were analysed. Other parameters were added to the model as normalisation constants.

All computed non-linear regression models were tested for significance of the single parameter estimates. Only models with parameter estimates that were significantly different from zero were included into the subsequent test procedure.

Two information criteria based methods were used to determine the model that best described the experimental data. Since the range of possible formulas to describe the data set includes models with different numbers of parameters, a simple coefficient of determination-based method is not sufficient. More accurate indicators for a discrimination of regression models are the Akaike information criterion (Akaike 1974) and the Schwarz/Bayesian information criterion (Schwarz 1978). Both the Akaike (AIC) and the Schwarz/Bayesian (S/BIC) information criterion were originally based on the maximized likelihood (L) of a model with a given number of parameters (k). Under the assumption that the model errors are normally distributed, L can be replaced with the residual sum of squares (RSS) divided by the number of observations (n):

$$AIC = 2k + n\ln(RSS/n) \quad (6)$$

$$S/BIC = k\ln(n) + n\ln(RSS/n) \quad (7)$$

The model associated with the smallest values of AIC and S/BIC is the most appropriate, with S/BIC being a more restrictive criterion on increasing parameter numbers.

The multivariate non-linear regression analysis was performed using a data set (n) of 210 AMR values. This number was achieved by testing 14 fish at each of the three temperatures at five different swimming speeds, respectively.

Statistics

The significance level for all statistical tests was $P < 0.05$. All calculated mean values were tested for significance using t-tests. Linear regressions of the allometric functions for standard metabolic rate and cost of transport as well as the power fits of the AMR to speed relationship were tested for overall significance of the data sets using F-statistics (Zar 1996). The F-test was performed by comparing a global model where slope is shared among the data sets with a model where each dataset gets its own slope, i.e. by comparing the pooled residuals of both discrete regressions to the regression residuals of the combined data set. Additionally, analysis of variation in the fitted slopes and intercepts across temperatures were computed in SPSS by using analysis of covariance (ANCOVA).

Results

The standard metabolic rate was directly measured in vendace at rest for 15°C. These values were compared to the extrapolated values from the swimming experiments at

this temperature. The two allometric regressions (Eq. 2, Fig. 1) were not significantly different (F-test: $F = 1.53$; $P = 0.22$). They showed similar intercepts, 0.0014 and 0.0017 (ANCOVA: $F = 0.79$; $P = 0.38$), and similar slopes, 0.77 and 0.71 (ANCOVA type III SS: $F = 0.70$; $P = 0.41$), for the direct and the estimated SMR values, respectively. It was therefore sufficient for further evaluations to use the estimates from the indirect method to compare the SMR at different temperatures. SMR increased with temperature (Fig. 3, Table 1) showing a significant difference between the regressions for 4, 8 and 15°C ($P < 0.01$ for all F-tests). The scaling exponents for these fits decreased with increasing temperature ($b_{SMR(4°C)} = 0.94$, $b_{SMR(8°C)} = 0.84$, $b_{SMR(15°C)} = 0.71$), showing significantly different slopes between 4 and 8°C (ANCOVA type III SS: $F = 6.34$; $P = 0.02$) and 8 and 15°C (ANCOVA type III SS: $F = 6.80$; $P = 0.02$).

For a comparison of the active metabolic rate at all speeds, the data of all individual fish were corrected for size with the respective temperature specific mass exponents from the SMR regressions. AMR and net AMR data are presented in Fig. 2 for all temperatures. While AMR increased significantly with temperature ($P < 0.01$ for all F-tests), net AMR did not differ significantly between 4 and 8°C ($F = 0.89$, $P = 0.45$). The individual speed exponents (c) of the AMR showed no clear trend with temperature, varying between 1.7 and 3.7 for all temperatures and body masses (Eq. 3). The AMR of every single speed at 15°C was further tested against the SMR from the direct measurements. No significant difference was found between the regressions for the SMR and the active metabolic rate at 0.5 ($P = 0.77$), 0.75 ($P = 0.34$) and 1.0 BL s^{-1} ($P = 0.25$). Only the swimming metabolism at 1.5 ($P < 0.01$) and 2.0 BL s^{-1} ($P < 0.001$) was significantly higher compared to the standard rate. A similar test for the other two temperatures was not possible because of the lack of independent SMR measurements.

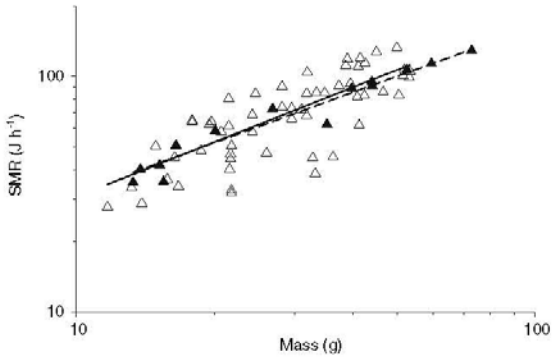

Fig. 1 Relationship between standard metabolic rate and body mass at 15°C. *Open triangles* represent the direct SMR measurements (regression indicated by *solid line*: 5.19 $M^{0.77}$) and *filled triangles* represent the extrapolated SMR values from the swim-tunnel tests (regression indicated by *dashed line*: 6.17 $M^{0.71}$). Regression lines were fitted by least-squares regression according to SMR = aM^b (Eq. 2). Note: double-logarithmic plot

The ratio of standard metabolism to total swimming costs at optimum speed was calculated for each temperature. Mean percentages were 46.4 ± 4.5, 68.6 ± 3.9 and 53.8 ± 5.9% at 4, 8 and 15°C, respectively. The differences were significant ($P < 0.01$ for all t-tests), but no trend with temperature was observed.

The swimming velocity associated with minimum energetic costs per unit distance, i.e. optimum speed (U_{opt}), showed no trend with temperature and only increased slightly with body mass at 4 and 8°C but not at 15°C. Mean relative optimum speeds were 2.4 ± 0.4, 2.0 ± 0.2 and 2.8 ± 0.9 BL s^{-1} at 4, 8 and 15°C, respectively. The minimum amount of energy needed by a fish to swim a unit distance (COT) increased with temperature and body mass (Fig. 3, Table 1). The regressions differ significantly between 4, 8 and 15°C ($P < 0.01$ for all F-tests). The slope of the relationship decreased with increasing temperature ($b_{COT(4°C)} = 0.81$, $b_{COT(8°C)} = 0.80$, $b_{COT(15°C)} = 0.70$), but the difference was only significant between 4 and 15°C (ANCOVA type III SS: $F = 8.10$; $P = 0.01$). The energy

Table 1 Regression analysis results for the allometric relationships (Eq. 2) for SMR, COT and net COT at all three temperatures

T (°C)	Constant	Estimate	SE	P	r^2
SMR (J h^{-1})					
4	a	1.125	0.246	<0.001	0.992
	b	0.944	0.056	<0.001	
8	a	2.285	0.855	0.020	0.975
	b	0.843	0.103	<0.001	
15	a	6.175	1.081	<0.001	0.994
	b	0.712	0.046	<0.001	
net COT (J m^{-1})					
4	a	0.002	0.000	0.004	0.985
	b	0.831	0.072	<0.001	
8	a	0.001	0.000	0.007	0.982
	b	0.784	0.086	<0.001	
15	a	0.006	0.001	<0.001	0.991
	b	0.565	0.054	<0.001	
COT (J m^{-1})					
4	a	0.003	0.001	0.002	0.987
	b	0.810	0.065	<0.001	
8	a	0.004	0.001	0.011	0.980
	b	0.797	0.092	<0.001	
15	A	0.008	0.003	0.011	0.980
	B	0.689	0.087	<0.001	

Estimate, standard error (SE) and P-value are given for each parameter

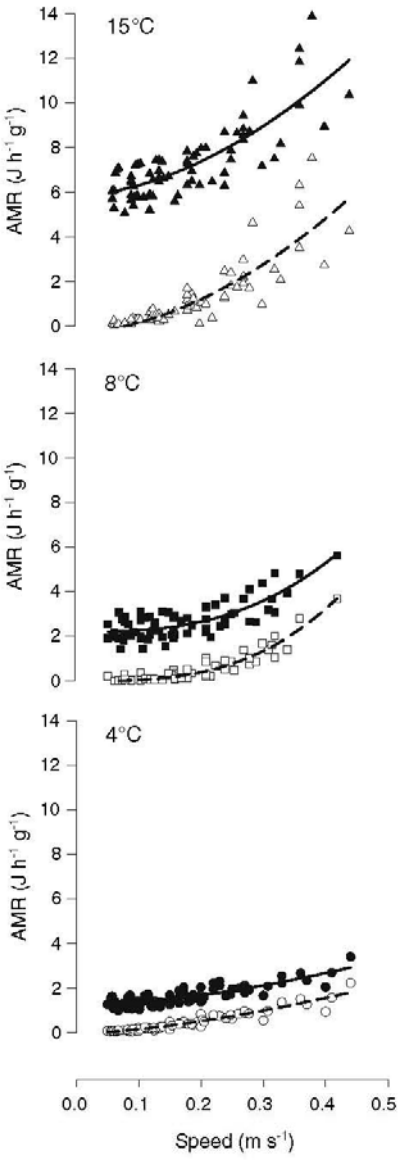

Fig. 2 Relationship between mass-corrected active metabolic rates and absolute swimming speed at 4, 8 and 15°C shown at the same scales. *Filled symbols* (*solid lines*) represent the total swimming costs (AMR) and *open symbols* (*dashed lines*) the net costs (net AMR). Regression lines were fitted by least-squares regression according to $AMR = a + bU^c$ (Eq. 3)

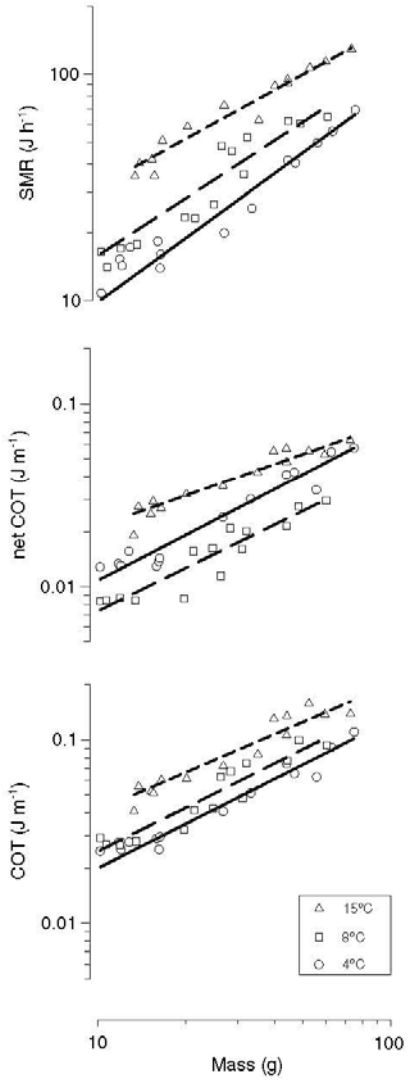

Fig. 3 Allometric relationships (Eq. 2) for the standard metabolic rate and the total and net minimum energetic costs per unit distance (COT, net COT) at 4, 8 and 15°C. *Triangles* (*short-dashed lines*) represent the 15°C, *squares* (*long-dashed lines*) the 8°C and *circles* (*solid lines*) the 4°C data. The regressions differ significantly between the temperatures for all three metabolic characteristics. The respective values for a and b are given in Table 1. Note: double-logarithmic plots

needed for locomotion only without the standard metabolism (net COT), showed a slightly different variation with temperature (Fig. 3, Table 1). The net COT was significantly lower in 8°C compared to 4°C and highest at 15°C ($P < 0.01$ for all F-tests).

The following energetic model was designed by multivariate non-linear regression analysis to predict active metabolic rates in vendace based on the presented selection criteria (Table 2):

$$\mathrm{AMR} = aM^b \exp(\mathrm{d}T) + eM^b U^c, \quad (8)$$

where AMR is the active metabolic rate (J h^{-1}), M is body mass (g), T is temperature (°C) and U is swimming speed

Table 2 Non-linear regression models for the active metabolic rate, ordered by the number of parameters included into the model (k) and the corresponding degrees of freedom (df)

Model	k	Df	r^2	RSS	AIC	S/BIC	NS
$AMR = aM^bU^c \exp(dT)$	4	206	0.903	38794	1104.0	1117.4	–
$AMR = aM^b \exp(dT) + eM^bU^c$	**5**	205	**0.916**	**33772**	**1076.9**	**1093.6**	**–**
$AMR = aM^b \exp(dT) + eM^bU^c \exp(dT)$	5	205	0.914	34440	1081.0	1097.7	–
$AMR = aM^b \exp(dT) + eM^fU^c$	6	204	0.932	27318	1034.3	1054.4	e
$AMR = aM^b \exp(dT) + eM^fU^c \exp(gT)$	7	203	0.932	27307	1036.2	1059.7	e, g

Given further are the coefficient of determination (r^2), residual sum of squares (RSS), Akaike information criterion (AIC), Schwarz/Bayesian information criterion (S/BIC) and non-significant parameter estimates (NS). A model was rejected if any of the estimated parameters was not significant. Out of the remaining models the one associated with the lowest AIC and S/BIC values was selected (bold)

(BL s^{-1}). Estimates of the constants a, b, c, d and e are given in Table 3. Insertion of the parameter estimates delivers the following formula ($r^2 = 0.92$):

$$AMR = 0.82M^{0.93}\exp(0.07T) + 0.43M^{0.93}U^{2.03}, \quad (9)$$

All other models either produced non-significant estimates of one of the parameters or showed higher AIC and SBIC values. The proposed formula represents the sum of standard plus net activity costs, where temperature is only related to the standard metabolism. Adding another temperature dependency to the activity term resulted in non-significant parameter estimates. Mass influenced standard and activity costs, but the mass exponent is the same for both since two exponents apart also resulted in non-significant parameter estimates (Table 2).

Discussion

The method of extrapolating to zero speed from activity measurements to estimate the SMR has been discussed controversially. The validity of this method has been questioned for reasons of potential overestimation (Videler and Nolet 1990; Dewar and Graham 1994) or underestimation (Forstner and Wieser 1990). However, there is also experimental validation for the method (Brill 1987). Moreover, two different fits have been used to describe the AMR to speed relationship, an exponential and a power function, which result in unequal estimates of the SMR (Pettersson and Hedenström 2000; Korsmeyer et al. 2002). In this study, the power fit constantly resulted in higher SMR values and showed higher correlation coefficients for the individual as well as the mass-specific AMR to speed data at all temperatures compared to the exponential fit. Moreover, the data presented here also support the accuracy of extrapolating to zero speed by applying the power fit, because the regressions of the direct SMR measurements and those from the swim tests showed no significant difference, suggesting that the power fit gives reliable estimates of the SMR. A comparison with data from a review by Clarke and Johnston (1999) shows that the calculated SMR for vendace are in good accordance with standard rates found in other salmonid species. The mean resting metabolic rate for a 50 g salmonid at 15°C is given by 0.231 mmol h^{-1} (Clarke and Johnston 1999, Table 1), which corresponds to an energy consumption of 104.9 J h^{-1} using the same oxycaloric value as in our study (14.2 mgO_2^{-1}, Hepher 1988). For a vendace of 50 g at this temperature, the regressions for the direct SMR and the extrapolated SMR measurements predict a metabolic rate of 106.5 J h^{-1} and 100.1 J h^{-1}, respectively.

The aerobic swimming performance of vendace is generally characterised by high ratios of standard to activity costs. The increase in metabolic costs when swimming at speeds of up to one length per second is not significant compared to the standard metabolism. These constant levels of activity costs might be attributed to the fact that the fish depend on compensatory movements by their pectoral and dorsal fins to keep balance when swimming at low speeds (Videler 1993; Hammer and Schwarz 1996). The phenomenon of constant metabolic rates at low swimming speeds has already been reported earlier (Forstner and Wieser 1990), but it is remarkable that the energetic costs remain relatively constant in vendace over such a wide range of swimming speeds. Even when swimming at optimum speed, standard costs account for up

Table 3 Statistical results of the non-linear regression according to the model $AMR = aM^b \exp(dT) + eM^bU^c$. Estimates and standard errors (SE) are given for each parameter

Estimate		SE
a	0.82	0.130
b	0.93	0.028
c	2.03	0.362
d	0.07	0.006
e	0.43	0.134

to 2/3 of the energy expenditure. This comparison shows that the SMR makes up a great proportion of the total swimming costs and points out the low energetic costs needed for propulsion. Claireaux et al. (2006) investigated sea bass and found similar ratios of standard to total metabolic costs at U_{opt} (55–60%) and similar optimum speeds (0.32 and 0.52 cm s^{-1} at 7 and 14°C, respectively) compared to vendace (0.28 and 0.41 cm s^{-1} at 8 and 15°C, respectively). Optimum speeds for various salmonids of different size classes at 12–15°C reviewed by Videler (1993) ranged from 0.8 BL s^{-1} in *Coregonus artedii* (Bernatchez and Dodson 1985), to 2.8 BL s^{-1} in sockeye salmon (Brett 1964). The calculated mean optimum speeds for vendace, 2.0 to 2.8 BL s^{-1}, lie at the upper end of this range, but other studies have calculated even higher optimum swimming speeds, for instance, Dabrowski et al. (1989) predicted U_{opt} of up to 4 BL s^{-1} for juvenile vendace.

Weihs (1973) predicts that a fish would swim at its optimum speed to maximize the distance covered per unit energy, when the energy needed for propulsion equals that for the standard metabolism, i.e. when SMR accounts for half of the total active costs, with corresponding optimum speeds of 1–2 BL s^{-1}. The 50% value is close to those found at 4 and 15°C in vendace, but the respective mean optimum speeds were distinctly higher (2.4 and 2.8 BL s^{-1}) then predicted by Weihs (1973). This suggests low net activity costs that enable the fish to swim at high speeds covering longer distances when doubling the standard energy expenditure per unit time.

The optimum swimming speed and the energetic costs at this speed have high ecological relevance, because free-ranging fish generally swim close to this speed during routine movements such as foraging (Ware 1978; Weihs and Webb 1983; Webb 1991; Videler 1993; Dewar and Graham 1994; Bejan and Marden 2006). It has been argued, that the optimum speed might be limited to long-distance migration and foraging (Steinhausen et al. 2005). Moreover, the optimum speed is influenced by the food concentration during foraging (Muir and Newcombe 1974), the direction of foraging (Tanaka et al. 2001) as well as the mode of swimming (Steinhausen et al. 2006). This implies that optimum speeds as calculated here are ecologically relevant only for long-term foraging under steady swimming conditions. However, long-term and steady foraging seems to be the most important swimming condition for the pelagic vendace in an oligotrophic lake under low food concentrations. For instance, Dabrowski et al. (1989) showed that juvenile vendace had to spend most of the time per day foraging, possibly above 20 h, to keep a high daily growth rate of 4% at very low food concentrations.

The cost of transport is especially useful for the ecological evaluation of the swimming characteristics as it determines the foraging or cruising efficiency of a species. The low net energetic costs during swimming (net COT) in vendace at 8°C might be attributed to the slightly lower optimum speeds found at this temperature. However, the power fits of the net AMR did not differ significantly between 4 and 8°C. This suggests that the energy needed for propulsion is similar at these temperatures, which roughly represent the year-round population depth of this species in its natural habitat (Helland et al. 2007).

The multivariate regression analysis of the metabolic rate in vendace explains 92% of the variance in total swimming costs. This indicates that body mass, temperature and swimming speed are adequate variables for modelling the energetic costs during swimming. Although it can be questioned if swimming tests from the laboratory are transferable to natural swimming conditions and ecological implications derived from these tests have to be made cautiously (Plaut 2001; Nelson et al. 2002), it is a reliable method in fish eco-physiology to describe the general effects of the investigated variables on the swimming performance and compare it to other laboratory-derived principles.

The mean mass exponent (0.93), estimated for the standard and activity metabolism by the multivariate regression analysis, is significantly higher than the ¾-power or other fish specific relationships, although the mean scaling dependency of the SMR for all three temperatures (0.83) is within the range recorded for other fishes, most commonly 0.8 (Winberg 1961; Clarke and Johnston 1999) and 0.88 (White et al. 2006). In vendace, the activity metabolism thus tends to show higher exponents compared to the standard metabolism. This is in accordance with Brett and Glass (1973), who measured scaling relationships of 0.99 for AMR and 0.88 for SMR in Sockeye salmon. Two separate scaling relationships were not considered in the model, since the regression analysis then resulted in non-significant parameter estimates (see Table 2). However, all tested models where a specific mass exponent for the SMR and AMR were included, gave significantly higher values for the activity compared to the standard metabolic rate.

The deviation from the ¾-power might also be explained by the higher mass exponents generally found for ectothermic compared to endothermic animals. McNab (2002) revealed in his meta-analysis of scaling relationships for various vertebrate taxa that ectotherms have higher exponents (0.71–1.06) than endotherms (0.67–0.75). Moreover, pelagic animals seem to have generally steeper metabolic scaling relationships than non-pelagic species (Glazier 2005). For instance, pelagic fish that are active swimmers show steeper exponents for muscle-enzyme activities than benthic fish (Childress and Somero 1990; Somero and Childress 1990) and it has been suggested that

larval fish hatched from pelagic eggs exhibit higher exponents than those hatched from benthic eggs (Oikawa et al. 1991).

The different mass exponents found for the SMR at 4, 8 and 15°C suggest a temperature dependency of the scaling relationship itself. This is in accordance with many other experimental studies where temperature was the major extrinsic factor altering the exponent of scaling relationships (Glazier 2005). It can therefore be assumed that the influences of temperature and body mass are linked to each other and do not independently affect the metabolic rate. Regarding the observed mass dependencies for the standard metabolism and the cost of transport at optimum speed, we can further assume that the scaling relationship varies not only with temperature but also with activity level. This pattern has already been described in other fish studies (Brett 1965; Robinson et al. 1983; Herrmann and Enders 2000) and it is a common rule observed in many vertebrate groups (Nagy 2005). Such dependencies of the scaling exponent cannot be considered when using universal exponents like suggested for the "metabolic theory of ecology" (West et al. 1997; Brown et al. 2004).

Exponential temperature coefficients in fish typically range from 0.05 to 0.10 (Jobling 1994), corresponding to Q_{10} values of 1.65–2.70 ($Q_{10} = e^{(a*10)}$). Clarke and Johnston (1999) suggest a mean value of 2.35 for the resting metabolism in teleosts, but White et al. (2006) computed a mean Q_{10} of 1.65 for 82 fish species. Other species-specific Q_{10} include values from 2.1 for walleye (Beamish 1990) to 2.8 for bream (Hölker 2006). The temperature coefficient (0.07) for vendace corresponds to a Q_{10} of 2.11, which is thus an intermediate value for fishes. The regression analysis revealed that temperature did only significantly affect the standard but not the net active metabolic rate. This effect is also apparent when the net cost of transport is considered, which showed no clear trend with temperature. The same lack of a clear thermal effect on net COT has been observed in sea bass (Claireaux et al. 2006), however, examples from other species suggest that swimming costs might be influenced by water temperature (Johnston and Temple 2002). Besides this, swimming performance may be influenced by water temperature in other than a linear form, for instance by optimizing processes, like it has been proposed for maximum swimming speed (Myrick and Cech 2000; Lee et al. 2003). Which of the swimming characteristics are subject of optimizing processes remains unclear. Besides optimum or maximum swimming speed, the energy expenditure per unit distance (COT) might also be subjected to selection pressures, since COT determines the foraging or cruising efficiency of a species. Clearly, these assumptions remain speculative and overreach the scope of this paper. However, the link between swimming performance and its ecological function represents an interesting field of future research to better understand the ecological implications of swimming characteristics in fish.

Speed exponents in fishes are generally assumed to range from 2 to 3 (Videler and Nolet 1990; Wieser 1991). Based on hydrodynamical considerations, values of 2.5 and 2.8 have been suggested for fish swimming under laminar or turbulent flow conditions, respectively (Webb 1975; Alexander 2005). The speed exponent contains information on the efficiency of aerobic swimming (Webb 1993). Higher speed exponents result in steeper power fits and represent lower swimming efficiencies. It has been shown that high-drag morphs have higher values compared to low-drag fishes (Pettersson and Hedenström 2000) and that differences in swimming efficiency can be explained by morphological characteristics (Ohlberger et al. 2006). Among various teleost fishes a mean value of 2.3 has been computed (Beamish 1978). Other studies reported exponents of 2.44 for sea bass (Claireaux et al. 2006), 2.53 for carp and 2.23 for roach (Ohlberger et al. 2006). Although values smaller than 2.0 have also been measured (Videler 1989), the mean speed exponent calculated for vendace (2.03) is at the lower end of the reported range. This suggests a fairly good swimming efficiency of vendace compared to many other fish species. Foraging at high speeds under low energetic costs might be necessary for a sufficient food capture in this pelagic fish species that is subjected to low prey densities in its natural habitat (Schulz et al. 2003).

Taken together, this study shows that net activity costs in vendace are low and that aerobic swimming performance is mainly dominated by the standard metabolic rate, making it very efficient in foraging with high optimum swimming speeds. Our analysis shows that temperature, swimming speed and body mass are appropriate variables for modelling the energetic costs of swimming. It was found that temperature mainly affects the standard but not the activity metabolism in this species. Finally, it was argued that the heterogeneity of the mass dependencies and the integrative effects of temperature and body mass on the metabolic rate do not support a universal scaling relationship.

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Paper III

III

Temperature-related physiological adaptations promote ecological divergence in a sympatric species pair of temperate freshwater fish, *Coregonus* spp.

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Temperature-related physiological adaptations promote ecological divergence in a sympatric species pair of temperate freshwater fish, *Coregonus* spp.

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Summary

1. Understanding the ecological forces that are responsible for the evolution and coexistence of closely related sympatric species is a major interest in evolutionary ecology. In a freshwater system context, flocks or pairs of sympatrically occurring fishes are commonly studied to identify causes and mechanisms of ecological divergence and speciation.

2. Whereas habitat and diet segregation along the pelagic–benthic axis is frequent in many species pairs in temperate freshwaters, two pelagic planktivores coexist in the post-glacial Lake Stechlin, Germany (common vendace, *Coregonus albula* and the endemic Fontane cisco, *C. fontanae*). Accordingly, disruptive selection on traits not related to feeding may have contributed to divergence. Since both species differ slightly in their vertical distribution, species-specific physiological adaptations related to temperature are likely.

3. We investigated standard and active metabolic rates (AMR) over the range of environmental temperature, which both species experience naturally (4–15 °C). Eighty-four specimens of 7–22 cm length were exposed to five relative flow velocities between 0·5 and 2·0 body lengths per second (BL s^{-1}) in swim tunnel respirometers.

4. Fontane cisco showed a reduced standard metabolic rate (SMR) compared to vendace over the entire temperature range. Further, activity metabolism was associated with lower energetic costs in Fontane cisco at 4 °C, but higher costs than in vendace at 8 °C and 15 °C. Total metabolic costs when swimming at 2 BL s^{-1} were higher for vendace at < 8 °C, but higher for Fontane cisco if temperature exceeds 8 °C.

5. The results indicate that species-specific physiological adaptations contribute to ecological divergence, thus preventing competitive exclusion between the pelagic coregonids of Lake Stechlin. The existence of a vertical gradient in water temperature was probably a crucial factor for the evolution of both competing temperature-related physiological strategies.

Key-words: ecological divergence, metabolic adaptation, sympatric speciation, temperature

Introduction

Divergent or disruptive natural selection arising from differential resource use, competitive intraspecific interaction or ecological opportunity causes phenotypic divergence and is hypothesized to be a major cause of speciation (Schluter 2000; Coyne & Orr 2004; Dieckmann *et al.* 2004). Divergent natural selection leads to local adaptation of subpopulations by evolving traits that provide an advantage under specific environmental conditions. Therefore, local adaptation plays a crucial role in generating phenotypic and population divergence in sympatry (Turelli, Barton & Coyne 2001), thus providing a mechanistic ecological understanding of the process of sympatric speciation (reviewed in Orr & Smith 1998; Bolnick & Fitzpatrick 2007). However, since the speciation history cannot be directly studied, it is fundamental to investigate which traits may be exposed to divergent natural selection and which ecological conditions promote diversification of these traits.

Sympatric species pairs have been described in several groups of teleost fishes from post-glacial temperate freshwater

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lakes, for example, in sticklebacks (*Gasterosteus* spp.), smelts (*Osmerus* spp.), charrs (*Salvelinus* spp.), and whitefish and ciscoes (*Coregonus* spp.) (see Taylor 1999). Some of these species pairs provide evidence for the process of sympatric speciation (e.g. Gislason *et al.* 1999; Knudsen *et al.* 2006), driven by competition for resources or habitats (Robinson & Wilson 1994; Schluter 1996). The common situation is the occurrence of limnetic–benthic species pairs that diverged into distinct ecotypes or species by exploiting either benthic food in profundal or littoral habitats, or planktonic food in pelagic habitats (e.g. Lu & Bernatchez 1999; McKinnon & Rundle 2002; Knudsen *et al.* 2006). The ecological segregation is supported by differences in morphological features, mainly related to the feeding process (Skúlason & Smith 1995). In contrast, in a few systems such as the North American Lake Superior, multiple pelagic planktivores coexist in sympatry without further benthic–pelagic segregation (Smith & Todd 1984). The ecological forces that are responsible for the evolution and coexistence of these competing populations remain unclear.

The co-occurrence of two pelagic planktivorous coregonid species is also described in the deep and oligotrophic, post-glacial Lake Stechlin (Germany), where the common European vendace (*Coregonus albula* (L.)) lives in sympatry with the endemic dwarf-sized Fontane cisco (*Coregonus fontanae* (Schulz & Freyhof)). The two species are easily distinguished by their differential spawning times, with vendace spawning in winter and Fontane cisco spawning from spring to summer. Both species exhibit distinct morphological characteristics, for instance, in the number of scales in the lateral line, body depth at dorsal-fin origin or gill raker length of the first gill arch (Schulz & Freyhof 2003). The evolution of this species pair by sympatric speciation has been suggested from mtDNA and microsatellite analyses (Schulz *et al.* 2006). Both species perform regular diel vertical migrations by ascending from their deepwater daytime habitat into shallower water layers during night-time (Helland *et al.* 2007, Mehner, Kasprzak & Hölker 2007). However, Helland *et al.* (2007) found that the average night-time population depths differed between the species, with Fontane cisco being found about 6 m deeper in the water column than vendace all over the year. Despite the fact that the weak depth segregation within the pelagic area may facilitate a depth-related feeding divergence, diet compositions of both species were rather similar with a clear dominance of planktonic food (Helland *et al.* 2008). Therefore, exploitative competition between the coregonids can be assumed to be high, and habitat segregation does not contribute substantially to competition avoidance. Rather, the strong correspondence between vertical fish distribution and the seasonally varying temperature gradient in Lake Stechlin (Mehner *et al.* 2007) suggests that metabolic adaptations exist that foster the coexistence of the coregonids in slightly differing thermal habitats.

The relation of the standard metabolism to temperature has been studied extensively and it was found earlier that temperature is the most important environmental factor if the standard metabolism in fish is considered (Jobling 1994). Furthermore, temperature-related swimming capacity is considered as a main characteristic determining survival in many species due to its impact on food capture, reproduction, predator avoidance and habitat shifts (Videler 1993; Plaut 2001). The swimming physiology is thus of high ecological importance and presumed to be subjected to selection pressures that enhance evolutionary fitness (Reidy, Kerr & Nelson 2000; Arnott, Chiba & Conover 2006). Thus, when fish are exposed to long-term temperature changes they can obtain optimal performance by altering their swimming physiology either by acclimation or adaptation (see Guderley & Blier 1988) and it has been suggested that metabolic rate differences between populations of one species reflect adaptations to specific environmental conditions (Álvarez, Cano & Nicieza 2006). Accordingly, we tested whether the swimming metabolism of the coexisting coregonid species differed over the temperature range to which the species are naturally exposed. By measuring standard and active metabolic rates (AMR), the costs of living in differential thermal habitats could directly be compared between the species. We assumed that a temperature threshold for similar-sized specimens should exist, at which the metabolic advantage switches from one species to the other, thus predicting the species-specific depth distribution along a temperature-depth gradient. Such a correspondence between field observations and experimental results would bridge the gap in our understanding on how divergent natural selection conditions directly influence the physiology-based competitive strengths of fish populations and thus may contribute to the evolution of two competing temperature-related physiological strategies.

Material and methods

FISH HATCHING AND MAINTENANCE

Fontane cisco and vendace were caught in Lake Stechlin (53°10′N, 13°02′E), Germany, by gill netting (15 mm mesh size) during their spawning times from April to June and from December to January, respectively. Ripe adults were striped and propagated artificially. Both species were raised under identical laboratory conditions. Larvae were hatched in glass aquaria at 4 °C and subsequently raised in circular basins (80 L). They were fed with rotifers, brine shrimp and commercially available dry food for fish larvae. Juveniles and adult fish were maintained in 500–1000 L basins at about 6–7 °C and fed with salmon dry food. Before experiments started, individuals of both species were held at the respective temperatures of 4 °C and 8 °C for at least 2 weeks and at 15 °C for at least 1 week after a preceding steady increase for 1 week of 1 °C per day. Feeding was interrupted 48 h prior to experiments in order to avoid elevated oxygen consumption rates due to specific dynamic action. Specimens of the investigated dwarf-sized Fontane cisco (n = 42) and vendace (n = 42) were measured as 3·6–37·4 g (7–16 cm) and 10·2–75·0 g (10–22 cm), respectively.

EXPERIMENTAL DESIGN

The respirometer design and data analysis are described in detail by Ohlberger, Staaks & Hölker (2007). Experiments were conducted with two differently sized Brett-type tunnel respirometers. The small

Table 1. Statistical results for the regression analysis of the standard metabolic rate ($J\ h^{-1}$) to body mass (g) relationship according to $SMR = \alpha \times M^{\beta}$ for Fontane cisco and vendace at 4 °C, 8 °C and 15 °C. Given are the estimates and standard errors (SE) for parameters α and β as well as the *P*-values of the ANCOVA test for significance between the species

	Fontane cisco		Vendace		ANCOVA (*P*-values)	
Temperature (°C)	α (± SE)	β (± SE)	α (± SE)	β (± SE)	Slope	Intercept
4	1·12 (± 0·14)	0·85 (± 0·04)	1·13 (± 0·25)	0·94 (± 0·06)	0·001	–
8	2·58 (± 0·49)	0·71 (± 0·06)	2·27 (± 0·84)	0·84 (± 0·10)	0·105	0·018
15	6·97 (± 1·03)	0·61 (± 0·05)	6·18 (± 1·08)	0·71 (± 0·05)	0·886	0·002

and the large swim tunnel consisted of a measuring recirculation loop (25 L, 5 L), with a swimming section included (15 × 40 cm, 10 × 30 cm), an ambient tank surrounding the swimming section and a cooled reservoir (100 L, 60 L) for supply with aerated fresh water. Oxygen consumption rates of the fish during swimming were measured by automated and computerized intermittent-flow systems, where each phase of closed respirometry is followed by a ventilation phase to re-establish the preset oxygen level in the swimming section (see Ohlberger *et al.* 2005, 2007). The respirometers were placed in a climatic chamber and equipped with a chilling unit for fine-tuning of the temperature.

Swimming trials were conducted at three environmental temperatures (4 °C, 8 °C and 15 °C) with each fish swimming at five different relative speeds (0·5, 0·75, 1·0, 1·5 and 2·0 body lengths per second ($BL\ s^{-1}$)). To allow adaptation to experimental conditions, a velocity of 0·5 $BL\ s^{-1}$ was run for at least 24 h. Subsequently, velocities of 0·75, 1·0, 1·5 and 2·0 $BL\ s^{-1}$ were run for 8–16 h each, day and overnight. After the experiment, fish were removed from the respirometer, weighed immediately and a blank value, that is, microbial background respiration was determined. It accounted for up to 21% and 33% of the total respiration in the small and large respirometer, respectively. The fish cross-sectional area did never exceed 10% of the area of the swimming chamber.

DATA ANALYSIS

The acute oxygen consumption rate ($mg\ O_2\ h^{-1}$) of the fish was computed by fitting a linear regression to the decrease in oxygen saturation against time for each phase of closed respirometry. These values were corrected for microbial respiration and converted into energy consumption rates ($J\ h^{-1}$) using an oxycaloric value of 14·2 $J\ mg\ O_2^{-1}$. Data from the adaptation phase at the beginning of each experiment, in which the fish showed elevated oxygen consumption rates due to handling stress, were excluded from further calculation. The AMR for every single swimming velocity per individual was computed from the lowest 10% of all oxygen consumption rates. This was done to determine the minimum AMR without elevated consumption rates due to spontaneous activity or stress phases (see Ohlberger *et al.* 2007). A power function was used to describe the metabolic rate to speed relationship: $AMR = a + b \times U^c$, where AMR is the total AMR ($J\ h^{-1}$), U is swimming speed ($BL\ s^{-1}$), and a, b and c are constants. Constant a represents the estimated standard metabolic rate (SMR), that is, metabolic rate at zero speed, and c is the speed exponent. By differentiation of this equation with respect to U and subsequent zero-setting, the swimming speed associated with minimum energetic costs per unit distance, also referred to as optimum speed, was determined (Videler 1993). Calculated optimum speeds may exceed the range of investigated speeds due to this procedure. The estimated SMR values were plotted against body mass according to an allometric function: $SMR = \alpha \times M^{\beta}$, where SMR is metabolic rate at rest ($J\ h^{-1}$), M is body mass (g) and β is the mass exponent. The SMR regressions were compared by analysis of covariance (ANCOVA) for differences in slopes and intercepts and by the Johnson–Neyman technique (J–N Tech) to identify regions of non-significance (see White 2003).

The temperature-specific β value from the allometric function was subsequently used to standardize the total AMR values for body mass to evaluate the mass-corrected AMR to speed relationship. Moreover, subtracting the SMR from these AMR values delivered the mass-corrected net AMR at a specific temperature. The net AMR to speed relationship for all individual data points at one temperature was then fitted using the previously described power function without parameter a: net $AMR = b \times U^c$. The power fits were tested for overall significance between the data sets of the two species using *F*-statistics (Zar 1996). This test was performed by comparing the pooled residuals of both discrete regressions to the regression residuals of the combined data set.

To compare the total energy expenditure of both species with respect to temperature, we calculated the gross AMR per unit time at 4 °C, 8 °C and 15 °C based on the derived SMR and net AMR functions: gross $AMR = \alpha \times M^{\beta} + (b \times U^c) \times M$. The gross AMR was then plotted as a function of swimming speed, covering not only the measured but also the range of calculated optimum speeds, and as a function of mass, covering the investigated mass overlap between the species at all three temperatures.

Results

Fontane cisco had a significantly lower SMR compared to vendace at all three temperatures (Fig. 1; Table 1). At 4 °C the regressions differed in slope (ANCOVA type III SS: $F_{3,24} = 13{\cdot}67$, $P = 0{\cdot}001$) with a range of non-significance below 10·7 g (J–N Tech). At 8 °C and 15 °C, there were no significant differences in slopes but in intercepts (ANCOVA, 8 °C: $F_{2,25} = 6{\cdot}43$, $P = 0{\cdot}018$; 15 °C: $F_{2,25} = 12{\cdot}13$, $P = 0{\cdot}002$), showing no regions of non-significance (J–N Tech). Further, the scaling relationship of the SMR with body mass (slope, b) tended to be lower in Fontane cisco at all three temperatures (Table 1). The scaling exponent increased with decreasing temperature in both species, ranging from 0·61 to 0·85, and from 0·71 to 0·94 in Fontane cisco and vendace, respectively.

Optimum swimming speeds for all three temperatures, calculated from the individual AMR to speed relationships, ranged from 2·0 to 2·9 (mean ± SD = 2·6 ± 0·5) and from 2·0 to 2·8 (mean ± SD = 2·4 ± 0·4) $BL\ s^{-1}$ in Fontane cisco and vendace, respectively.

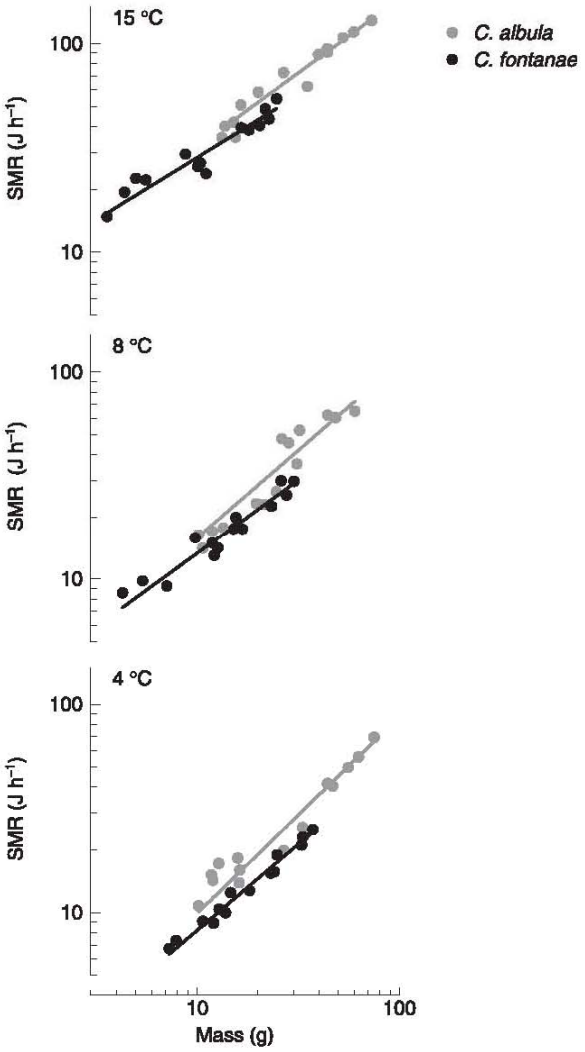

Fig. 1. Allometric relationships for the standard metabolic rate (SMR = $\alpha \times M^{\beta}$) of Fontane cisco (black) and vendace (grey) at 4 °C, 8 °C and 15 °C. The standard metabolic rate is lower in Fontane cisco at all three temperatures. At 4 °C the regressions differ significantly in slope, whereas at 8 °C and 15 °C there is a significant difference in intercepts. The slope of the scaling relationship (β) also tends to be lower in Fontane cisco at all three temperatures. The respective values for α and β as well as the ANCOVA tests are given in Table 1. Note: double-logarithmic plots.

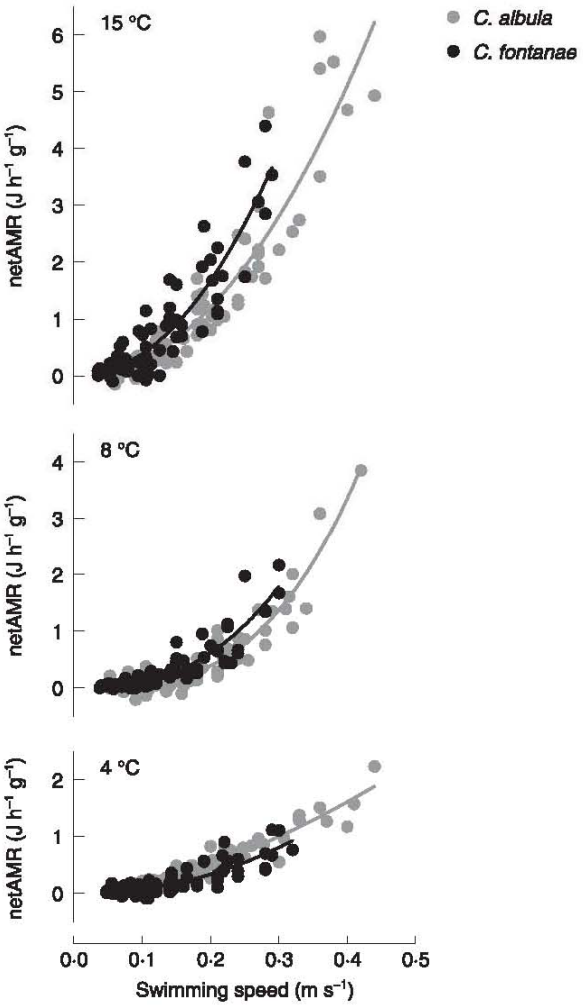

Fig. 2. Relationships between mass-corrected net active metabolic rate and absolute swimming speed (net AMR = $b \times U^{c}$) for Fontane cisco (black) and vendace (grey) at 4 °C, 8 °C and 15 °C. The net active metabolic rate (J h^{-1} g^{-1}) is significantly higher in Fontane cisco at 8 °C and 15 °C, but it is significantly lower at 4 °C. The respective values for *b* and *c* as well as for the *F*-statistics are given in Table 2.

The net AMR (J h^{-1} g^{-1}) of the Fontane cisco was higher at 8 °C and 15 °C, but lower at 4 °C compared to vendace (Fig. 2; Table 2). The differences between the data sets of the two species were significant at all three temperatures (*F*-test: $P < 0{\cdot}001$). The speed exponents (*c*), that is, the rates of increase in metabolic costs with increasing speed, varied with temperature in both species, but the calculated overall mean value was the same in both species ($c = 2{\cdot}3$).

The total energy expenditure per unit time during swimming (gross AMR) increased with speed, body mass and temperature. The relative total costs of the species compared to each other varied depending on temperature (Fig. 3). At 4 °C Fontane cisco had lower total costs than vendace over the entire speed range. At 8 °C and 15 °C Fontane cisco had lower costs at speeds up to 2 and 1·5 BL s^{-1}, respectively, but experienced higher energetic costs compared to vendace at swimming speeds above these values (Fig. 3).

Discussion

DIFFERENCES IN METABOLIC COSTS

Our study reports significant physiological differences in standard and swimming metabolism between two sympatric coregonid fishes. In the dwarf-sized Fontane cisco, standard metabolism was lower than in vendace over the whole temperature range tested. However, the net AMR in Fontane

Table 2. Statistical results for the regression analysis of the net active metabolic rate (J h^{-1} g^{-1}) to swimming speed (m s^{-1}) relationship according to net AMR = $b \times U^c$ for Fontane cisco and vendace at 4 °C, 8 °C and 15 °C. Given are the estimates and standard errors (SE) for parameters b and c as well as the F- and P-values of the F-test for significance between the species

	Fontane cisco		Vendace		F-test	
Temperature (°C)	b (± SE)	c (± SE)	b (± SE)	c (± SE)	F-value	P-value
4	10·70 (± 3·43)	2·15 (± 0·23)	7·345 (± 0·64)	1·66 (± 0·07)	17·19	< 0·001
8	41·61 (± 11·98)	2·62 (± 0·20)	57·76 (± 10·70)	3·11 (± 0·17)	11·99	< 0·001
15	49·91 (± 11·99)	2·11 (± 0·16)	34·35 (± 5·12)	2·08 (± 0·13)	11·72	< 0·001

cisco was lower than in vendace only at 4 °C, but meant higher costs at 8 °C and 15 °C. This diversification was corroborated by the estimates of total costs. At a temperature of 4 °C, Fontane cisco had lower total costs over the whole mass and swimming speed range due to low standard and active metabolic costs. At 8 °C and 15 °C, swimming was associated with higher total costs in Fontane cisco than in vendace at speeds above 2 and 1·5 BL s^{-1}, respectively. This means that the slope of the relationship between metabolic rate and temperature differs between the species pointing to the influence of temperature itself in dictating this slope through energetic trade-offs and evolutionary temperature adaptation. Consequently, these two diverging trends in standard and swimming metabolism with temperature suggest that Fontane cisco is better adapted to lower environmental temperatures compared to the co-occurring vendace.

The relatively high mass-scaling exponents found in both species deviate from the three-fourth power law (West, Brown & Enquist 1997), which might be explained by generally steeper metabolic scaling relationships in ectothermic compared to endothermic animals (McNab 2002). The finding that the exponent itself is temperature dependent is in accordance with many other experimental studies where temperature was the major extrinsic factor altering the exponent of scaling relationships, but the reason for such a temperature-dependence is not known (Clarke 2004). Since differences in mass exponents were significant between temperatures but not significant between species, this does not relate to the observed species-specific divergence in metabolism. The optimum swimming speeds (lowest costs) of Fontane cisco and vendace were found in the range of 2–3 BL s^{-1}. Dabrowski, Takashima & Law (1989) developed a bioenergetics model on coregonid fish growth and predicted optimum speeds for juvenile vendace of 1·8 to 4 BL s^{-1}. Generally, free-ranging fish swim close to optimum speed during routine movements like foraging (Videler 1993; Webb 1993). In subarctic Norwegian lakes, vendace activity was recorded by *in situ* stationary hydroacoustics, and swimming speeds of up to 3 and rarely even 4 BL s^{-1} were estimated during crepuscular or stronger light conditions (Gjelland *et al.* 2004). All these studies suggest that swimming speeds above 2 BL s^{-1} are common under natural conditions. Accordingly, the advantage of having lower total metabolic costs during foraging switches for similar-sized fish from Fontane cisco to vendace at a temperature threshold of *c.* 8 °C.

TEMPERATURE AS ECOLOGICAL RESOURCE

The prediction of a temperature threshold in relative metabolic advantage at *c.* 8 °C, which is based on the experimental results, can be compared with the experienced environmental temperatures at the average population depths of the fishes in Lake Stechlin over the seasonal course. According to catches with a midwater trawl in several water depths, the Fontane cisco population was never found in water layers with a temperature above 7·7 °C (Helland *et al.* 2007). In contrast, the average population depth of vendace was continuously in shallower water layers, suggesting that vendace experienced on average higher water temperatures. Accordingly, there is strong empirical evidence that the differences in vertical distribution between the two closely related coregonids are correlated to species-specific metabolic adaptations to slightly differing temperature ranges.

Early studies treated temperature as an ecological resource, and grouped fish into thermal guilds according to their preferred temperature (Magnuson, Crowder & Medvick 1979). Differences in depth distribution between fishes belonging to different thermal guilds corroborated that temperature gradients create spatial structures in vertical fish distribution (Rudstam & Magnuson 1985). If temperature and food availability were experimentally modified, the habitat choice of fish followed primarily by their temperature preference, and food was ranked second in the decision hierarchy (Krause, Staaks & Mehner 1998). Accordingly, the existence of vertical gradients in water temperature directly influences the physiology-based competitive strengths of fish populations (Jensen *et al.* 2006), and thus may contribute to the co-occurrence of competing species such as the two planktivorous coregonids in Lake Stechlin.

TEMPERATURE-RELATED METABOLIC ADAPTATIONS

The metabolic adaptation of Fontane cisco comprises two different physiological mechanisms. Standard costs are reduced over the whole temperature range. The standard metabolism includes the energy demand for many different processes thus representing the cost of living a particular lifestyle at a given temperature (Clarke 2003). In contrast to the SMR reductions, activity costs in Fontane cisco are also reduced at lower temperatures, but elevated at higher temperatures, as compared to vendace. This pattern might be explained by a

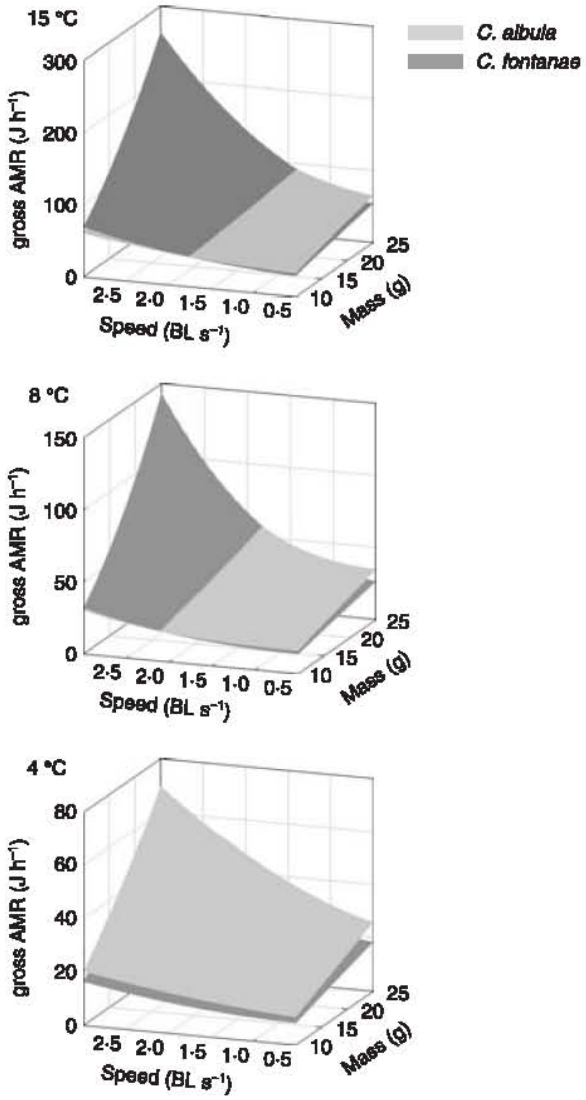

Fig. 3. Relationships of the total energetic costs (gross AMR) over swimming speed and body mass for Fontane cisco (dark grey) and vendace (light grey) at 4 °C, 8 °C and 15 °C. Gross AMR was calculated according to the previously derived functions for the SMR and the mass-corrected net AMR (gross AMR = $\alpha \times M^{\beta} + (b \times U^{c}) \times M$). All plots are presented on the same x and y scales, covering the measured plus the range of calculated optimum speeds, and the overlapping mass range of the species at all three temperatures. At 4 °C swimming costs are lower in Fontane cisco over the whole speed and mass range, whereas at 8 °C and 15 °C this energetic advantage is restricted to low swimming speeds. At the higher temperatures vendace is the stronger competitor during foraging at speeds above 2 BL s^{-1} due to the lower energetic costs.

trade-off mechanism in the physiological characteristics of the swimming musculature. For instance, thermal specialization optimizing performance at a particular temperature may be accompanied by a trade-off reducing the ability to respond to changing environmental temperature (Huey & Hertz 1984). In general, ectothermic organisms acclimate by adjusting physiological rates as well as their biochemical composition, thereby favouring the maintenance of function and capacity at a new acclimation temperature, but not necessarily at other temperatures (Guderley 2004). Important evolutionary responses to temperature change include modifications to membrane properties and intracellular milieu (Clarke 2003), temperature-dependent gene expression of key aerobic enzymes (Lucassen *et al.* 2006) and changes of the amount of mitochondrial enzyme or total mitochondrial density, which in turn affect standard metabolism and aerobic exercise capacity (see Pörtner *et al.* 2006). The consideration that temperature adaptation enhances evolutionary fitness in a new environment is based on the 'beneficial acclimation hypothesis' (Huey & Berrigan 1996). However, knowledge about the evolutionary significance of temperature acclimation and adaptation is still incomplete (see Clarke 2003).

ENERGETIC CONSEQUENCES

The standard and swimming costs of a fish are of high ecological importance, since they account for a great deal of the fish's energy budget (Jobling 1994; Hölker & Breckling 2002). Therefore, standard metabolism and swimming capacity are seen as a target of energy-reducing strategies and are presumed to be subjected to selection pressures that enhance evolutionary fitness (Reidy *et al.* 2000). Physiological adaptations of these metabolic rates might therefore be an adequate adaptive strategy to decrease energy losses for fishes in environments with low resource levels and high exploitative competition (Mueller & Diamond 2001). Correspondingly, the reduced metabolic costs at low temperature in Fontane cisco presumably compensate for energetic disadvantages associated with lower food densities in deeper water layers. In Lake Stechlin, the majority of zooplankton is concentrated in water layers close to the thermocline, and zooplankton biomass substantially declines with increasing water depths (Mehner, Hölker & Kasprzak 2005a). Accordingly, the occurrence of the dwarf-sized Fontane cisco in deeper layers than vendace has the consequence that the overall diet availability is reduced for this species. The lower energetic needs of this species in cooler waters may compensate for these disadvantages. If we calculate the energy expenditure for a 20-g fish swimming close to optimum speed at 2·5 BL s^{-1}, Fontane cisco need 19% less energy than vendace at a water temperature of 4 °C, but 16% more energy when swimming at 15 °C. The demand for energy intake, that is, for foraging, is reduced or enhanced accordingly. Svärdson (1979), based on his broad study of *Coregonus* spp. in Swedish lakes, hypothesized that being dwarf-sized might be an adaptation to living in deep layers with its associated cold environments and reduced food densities. The main purpose of such adaptations might be to save energy primarily for the production of large and relatively numerous eggs, a life-history feature typical for the order Salmoniformes to which the coregonids belong (Vila-Gispert, Moreno-Amich & Garcia-Berthou 2002).

IMPORTANCE FOR ECOLOGICAL DIVERGENCE AND SPECIATION

In freshwater fishes, there is ample evidence that phenotypic diversification and ultimately sympatric speciation are the outcome of divergent natural selection, most often driven by resource competition (Robinson & Wilson 1994; Schluter 1996). In the majority of sympatric freshwater species pairs, the common pattern of resource partitioning is found along the limnetic–benthic axes (e.g. Gislason *et al.* 1999; Knudsen *et al.* 2006). However, the process of diversification and adaptive radiation might also be facilitated by ecological opportunity (Schluter 2000), which is often found in newly colonized habitats (Skúlason & Smith 1995). Recent studies suggest that ecological opportunity in species-poor post-glacial lakes promotes adaptive diversification in fishes via disruptive selection mediated by intra-specific competition (Bolnick 2004; McKinnon *et al.* 2004).

In the post-glacial Lake Stechlin, genetics based on micro-satellites, mtDNA and AFLP data suggest that the evolution of vendace and Fontane cisco has occurred in sympatry (Schulz *et al.* 2006; K. Pohlmann, T. Mehner, unpublished data). However, in contrast to the benthic–limnetic diversification as found in other *Coregonus* or *Salvelinus* systems, the coregonids in Lake Stechlin are both pelagic, planktivorous feeders with weak diversification in feeding morphology (Schulz & Freyhof 2003). Accordingly, exploitative competition between the species is assumed to be intense. The metabolic adaptations as indicated in this study may facilitate coexistence since the two coregonid species can partition the pelagic zone into different thermal habitats to achieve energetic advantages over the competitor. This is corroborated by diet analyses of fishes from Lake Stechlin which demonstrated weak absolute differences between Fontane cisco and vendace, but a strong influence of depth of occurrence on the prey species composition (Helland *et al.* 2008). Thus, the metabolism-driven depth diversification is an ecological mechanism promoting reduced interspecific competition. The Fontane cisco strategy of lowered metabolism and dwarfism compensates for energetic disadvantages in deeper water layers compared to the pre-existing vendace strategy. Thus, we suggest that the existence of a vertical gradient in water temperature directly influences the competitive strengths of these fish populations and thereby contributed to the evolution of two competing temperature-related physiological strategies.

The reported divergence in metabolism may have contributed to speciation. Adaptations to different temperature regimes have been reported for sibling species in some taxa, for instance, scorpionflies (see Sauer, Vermeulen & Aumann 2003) and polychaetes (Kruse, Strasser & Thiermann 2004). Smith & Todd (1984) formulated the idea that the divergence and speciation in the Great Lakes ciscoes might be driven by steep environmental gradients of the lacustrine environment. Moreover, theoretical studies suggest that speciation along gradients may be a common process (Doebeli & Dieckmann 2003). It is interesting to note that sympatric species pairs of *Coregonus* occur in just two lakes in Germany, Lakes Stechlin and Breiter Luzin (Schulz *et al.* 2006), both of them being the deepest lakes in that region (Mehner *et al.* 2005b). Thus, environmental gradients may be steepest in these lakes, suggesting that species diversifications along the gradients are likely.

We do not state that a correlation between physiological traits and environmental variables provides evidence for evolutionary change *per se*, but infer adaptation as the most likely explanation for the pattern we observe in these closely related sympatric species (but see Garland & Adolph 1991). Further, as illustrated by Garland & Adolph (1994), two-species comparisons are believed to provide only weak evidence for evolutionary correlations. However, in the case of sympatric species pairs, no equally useful alternative approach based on multiple-species comparisons or incorporation of phylogenetic information is possible. Moreover, the two species investigated in this study provide exceptionally strong evidence for the role of natural selection in phenotypic divergence, because they co-exist in sympatry, have a short evolutionary history since speciation and show a huge niche overlap in most environmental dimensions. Random genetic drift and genetic differentiation due to the process of speciation itself that might have affected phenotypic traits cannot be ruled out as a cause of differentiation, but the clear relation of trait differences to environmental conditions strongly suggests a phenotype-environment association in this case.

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Paper IV

IV

Physiological specialization by thermal adaptation drives ecological divergence in a sympatric fish species pair

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Physiological specialization by thermal adaptation drives ecological divergence in a sympatric fish species pair

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ABSTRACT

Hypothesis: Divergence in thermal preference, in combination with temperature-related metabolic adaptations, promotes ecological specialization and co-existence of a sympatric species pair of coregonid fish.

Background: The coregonids show substantially lower divergence in diet composition than other sympatric fish pairs in temperate freshwater lakes, but segregate vertically within the pelagic area and differ in physiology with respect to metabolism.

Organism: Sympatric European vendace (*Coregonus albula*) and endemic dwarf-sized Fontane cisco (*C. fontanae*) from Lake Stechlin, Germany.

Methods: We determined the final thermal preferendum (FTP) of both species previously hatched and raised under identical laboratory conditions by using a shuttle-box system that allowed the fish to control body temperature through behavioural thermoselection.

Results: The species showed clearly different temperature selection behaviours. Vendace had a significantly higher FTP (9.0°C) than Fontane cisco (4.2°C). The FTPs comply with temperatures of minimum net swimming costs, suggesting that performance is optimized at the respective thermal preference of both species.

Conclusions: The correspondence of thermal preference, thermal performance functions, and thermal ecology shows that the species are adapted to different temperature regimes.

Keywords: *Coregonus* spp., divergence, sympatric species, temperature preference.

INTRODUCTION

Sympatric species pairs of temperate freshwater fish occupying postglacial lakes are commonly used as model systems to study the ecology of adaptive diversification. The adaptive

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speciation and radiation theory states that phenotypic divergence and speciation are ultimately the outcome of disruptive or divergent natural selection arising from differential resource use, competitive intraspecific interaction or ecological opportunity (Schluter, 2000; Coyne and Orr, 2004). Ecological opportunity is often found in newly colonized habitats like postglacial lakes, where sympatric species pairs are common (Skúlason and Smith, 1995; Day and Young, 2004). Many of these closely related sibling species show a clear ecological divergence (Schluter, 1996, 2000), which is required to avoid competitive exclusion and to enable co-existence (Coyne and Orr, 2004). Hence, much of the recent research in this field has focused on the role of ecological specialization in driving and maintaining divergence in sympatry.

Coregonines are among the most diverse freshwater fishes in temperate regions. Multiple forms or sympatric pairs within one genus are common in this group and some of the species have produced a considerable diversity in postglacial northern lakes (reviewed by Hudson *et al.*, 2007). Although indications for sympatric or allopatric diversification can hardly be teased apart, potential cases of ecologically based sympatric speciation from a single lineage have been identified (e.g. Lu and Bernatchez, 1999; Knudsen *et al.*, 2006; Schulz *et al.*, 2006). However, the speciation history cannot be directly studied by means of ecological field observations. Therefore, it is fundamental to identify trait differences and environmental conditions that promote a divergence in traits under disruptive selection, which might ultimately lead to evolutionary diversification.

Environmental temperature is a critically important factor for all ectothermic vertebrates. It has profound effects on almost all aspects of ectotherm behaviour and physiology, including individual performance, foraging ability, and fitness (Bennett, 1980; Huey, 1982; Videler, 1993; Huey and Berrigan, 2001; Bicego *et al.*, 2007). Hence, ectothermic vertebrates generally attempt to control body temperature within a species-specific range to optimize physiological processes and minimize disadvantageous temperature effects (Freidenburg and Skelly, 2004). Responses to changes or heterogeneity in environmental temperature occur on different time-scales (Hertz, 1981). Acute temperature adjustments are primarily achieved via behavioural thermoregulation (Cowles and Bogert, 1944; Bogert, 1949; reviewed in Casey, 1981). Individual acclimation on longer time-scales occurs via changes in enzyme expression, cell membrane modifications or alteration of the intracellular milieu (Hochachka and Somero, 1968; reviewed in Somero, 2004). Finally, on the time-scale of generations, evolutionary thermal adaptation may occur via changes in thermal performance functions (Angilletta *et al.*, 2006; Pörtner *et al.*, 2006). It is under debate, however, whether optimum temperatures of performance are subjected to strong selection pressures or are evolutionarily conservative (see Angilletta *et al.*, 2002). This may depend to a large extent on the abilities of an organism to acclimate to various temperatures and to control body temperature via thermoregulation, which varies considerably between different taxa and depends on the environmental conditions of the habitat.

Small poikilothermic fish are thermoconforming animals that are highly dependent on environmental temperature due to the high specific heat and thermal conductance of water as well as the efficient heat exchange between body and water (Stevens and Sutterlin, 1976). Consequently, in thermally heterogeneous environments, most fishes can only control body temperature and thus performance abilities by selecting the thermal microhabitat where physiological function is relatively efficient (Beitinger and Fitzpatrick, 1979; Angilletta *et al.*, 2006). Accordingly, the thermal preference of fish has been correlated with optima in various aspects of physiological performance (e.g. Brett, 1971; Kellogg and Gift, 1983; Hölker *et al.*, 2004) and fishes are generally believed to be well adapted to the environmental temperature of their natural habitat (Magnuson *et al.*, 1979; Johnson and Kelsch, 1998; Pörtner, 2002).

In the deep postglacial Lake Stechlin (Germany), two coregonid species co-exist in sympatry within the pelagic habitat, common vendace [*Coregonus albula* (L.)] and the endemic dwarf-sized Fontane cisco [*Coregonus fontanae* (Schulz and Freyhof, 2003)]. They are easily distinguished by spawning time and have been described by distinct morphological characteristics (Schulz and Freyhof, 2003). Based on genetic analyses, it was suggested that this species pair has evolved via sympatric speciation (Schulz *et al.*, 2006). Both species are pelagic planktivores that show only weak divergence with respect to diet composition (Helland *et al.*, 2008). This contrasts the commonly observed limnetic–benthic divergence of sympatric fish pairs in northern temperate lakes, which is based on the exploitation of different food resources (e.g. Lu and Bernatchez, 1999; Knudsen *et al.*, 2006). In Lake Stechlin, it has been found that the average night-time population depths differ between the species, with Fontane cisco being found a few metres deeper in the water column than vendace throughout the year (Helland *et al.*, 2007). This divergence in vertical distribution is associated with a difference in average experienced water temperature. Moreover, in an earlier study on the species' metabolism, we found temperature-related differences in standard and active metabolic rates, which showed that swimming performance is optimized at different temperature regimes in these two sister species (Ohlberger *et al.*, 2008).

In this study, we wished to determine whether the species pair from Lake Stechlin diverged with respect to the two species' temperature preference. Our hypothesis was that the thermal preferences of the species would correspond to the divergence in thermal ecology and thermal physiological performance. This was based on the 'co-adaptation hypothesis', which states that the sensitivity of thermal performance and thermal preference are closely co-adapted in ectotherms (Angilletta *et al.*, 2006). This assumption holds if the fitness of an organism is positively related to performance – that is, if the selection of temperatures that maximize performance compared with other temperatures implies fitness gains. Accordingly, a shift in the temperature dependence of metabolic costs should provide selective pressures for a corresponding shift in thermal preference. We thus hypothesized that the species would show differences in their temperature selection behaviour as a consequence of adaptation to different thermal microhabitats, with the deeper living Fontane cisco preferring lower temperatures than vendace. We investigated the thermal selection behaviour in a free-choice experiment in the laboratory using individuals of both species that were previously hatched and raised under identical conditions. To elucidate which metabolic characteristics (i.e. thermal performance functions) are optimized at the preferred temperatures, we analysed the temperature dependence of standard and activity costs as well as derived metabolic characteristics like total and net costs of transport. This analysis was carried out based on metabolic rates data from previous studies on the two species (Ohlberger *et al.*, 2007, 2008).

MATERIALS AND METHODS

Fish

Fontane cisco and vendace were caught by gill netting (15-mm mesh size) during their respective spawning times in Lake Stechlin (53°10′N, 13°02′E), Germany. Ripe adults were striped and propagated artificially. Larvae were hatched in 50-litre glass aquaria and subsequently maintained in 80-litre circular basins. They were fed with brine shrimp, rotifers, and commercially available dry food for fish larvae. Juveniles were transferred to

rectangular basins of 500–1000 litres and fed with salmon dry food. The fish were acclimated under a 12:12 h photoperiod to a temperature of 4°C for at least one month before the experiment began. Specimens of the investigated Fontane cisco and vendace measured 9–14 cm in total length.

Thermal preference determination

The thermal preferendum is operationally defined as the range of environmental temperatures in which an animal aggregates in a free-choice situation. In contrast to the acute thermal preferendum, which is selected as a short-term response and which is affected by previous acclimation temperature, the final thermal preferendum (FTP) is the temperature selected as a long-term response, which is unaffected by previous acclimation (Reynolds and Casterlin, 1979; Kelsch and Neill, 1990).

Experimental design

The experimental set-up we used was an electronically controlled shuttle-box system, which allowed the fish to control their body temperature by moving between two aquarium sections with a slight difference in water temperature (Neill *et al.*, 1972). The shuttle-box consisted of two interconnected compartments ($50 \times 50 \times 250$ cm and 600 litres in total) large enough to ensure normal swimming behaviour. A plastic panel with a centred hole of 10 cm diameter separated the compartments so that the fish could move freely between them. The aquarium was placed under a dark curtain to reduce disturbance of the fish and with light sources above it to control illumination. A constant temperature difference of 2 K (Kelvin) was established between the sections by using cooling coils and electric heaters (thermostat: Lauda UKT 1000). A computer controlled the measuring and regulating units with software for temperature regulation (Staaks, 1996). Thirty-two pairs of infrared transmitters and photocells continuously monitored the activity of the fish within the sections. Fish distribution was registered by the count of impulses at the photocells. The system was automatically cooled down or heated up in a dynamic manner according to the distribution of the fish between the sections. When fish preferred the warmer section, temperature in both compartments was increased and vice versa until the fish distributed evenly between the sections and the system's temperature stabilized.

Experimental procedure

Five experiments with six individuals each were performed on both species. Before an experiment, fish were maintained in the shuttle-box aquarium at the acclimation temperature of 4°C for one week to allow habituation to the system. Three fish were placed in each of the two compartments one week before the experiments began. Because coregonids are schooling fish, we used groups of six individuals and treated each of these groups statistically as one unit. After the start of an experiment, the fish usually controlled water temperature within one day. During the tests, fish were fed once a day with dry food at a random schedule. To avoid an accidental runaway of the system before fish had learned to behaviourally control the temperature, maximum speed of temperature change was limited to 2 $K \cdot h^{-1}$, maximum water temperature was set to 22°C, and minimum water temperature was limited by the cooling capacity to approximately 3°C. According to previous

experimental experiences (Staaks, 1996) and after the first test with Fontane cisco (group 8), an experimental period of 15 days was considered sufficient. After testing the first group of vendace (group 1), the experimental period for vendace was adjusted to about 25 days. Unfortunately, the controlling computer collapsed during two experiments so that test periods were a few days shorter in one vendace and one Fontane cisco group (groups 4 and 6), which nevertheless seemed to have gravitated to their final preference and were therefore included in the data analysis.

Data analysis

Temperature data were recorded at 10-min intervals and subsequently aggregated to daily mean values. To identify qualitative features of the individual recordings, an interval sequence approach was applied (Konstantinov and Yoshida, 1992). As a first step, kernel smoothing was applied using a loess smoother (Cleveland *et al.*, 1992) to remove noise from the time-series (Fig. 1). We used a Gaussian kernel, a smoothing window of 7 days, and an output step size of one hour. After this, segments with a temperature increment of less than 1 $K \cdot day^{-1}$ and a minimum duration of one day were regarded as stable periods, or if not as increasing or decreasing segments respectively. Variances were compared using the F-test and mean values using the Welch t-test, since homogeneity of variance was violated ($F = 9.64$; $df_1 = df_2 = 4$; $P = 0.0497$). Approximate normality of the samples was ensured graphically. The computations were performed using the data analysis system R (R Development Core Team, 2007) and the add-on package qualV (Jachner *et al.*, 2007).

Additionally, we analysed the temperature dependence of several metabolic characteristics (data in Ohlberger *et al.*, 2008) to detect physiological traits that are optimized at the respective final thermal preferences of the species. Standard and active metabolic rates, total swimming costs, and net costs of transport were plotted, for the three temperatures 4, 8, and 15°C, as a function of body mass and subsequently fitted according to a two-parameter power function ($a \cdot M^b$) (see Ohlberger *et al.*, 2007). These linear regressions were tested for overall significance of the data sets using F-statistics.

RESULTS

The two species differed clearly in their temperature selection behaviour (Fig. 1). Vendace generally showed multiple-phase behaviour. At the beginning of an experiment, these fish rapidly increased water temperature within a few days to 13–21°C and maintained these temperatures for 5–12 days. Only group 1 had a short intermittent period at lower temperatures before returning to about 15°C. Subsequently, vendace groups decreased water temperature to approximately 5–6°C and maintained these for a few days. Finally, the fish gravitated to an intermediate-temperature range between 7 and 11°C. Only group 2 immediately gravitated to a temperature range of 9–10°C after the initial high-temperature phase. All inter-phase periods were characterized by rapid temperature increases or decreases of up to 9 $K \cdot day^{-1}$. In contrast to vendace, Fontane cisco showed no distinct phases of temperature selection behaviour in most cases. All Fontane cisco groups usually selected water temperatures of about 4–5°C (i.e. similar to their acclimation temperature) over the entire course of the experiments. Only small variations were observed in two of the groups. Group 7 peaked to 9.9°C after a few days, but rapidly returned to the 4–5°C temperature range. Group 8 showed an intermediate increase to about 6°C, but returned to

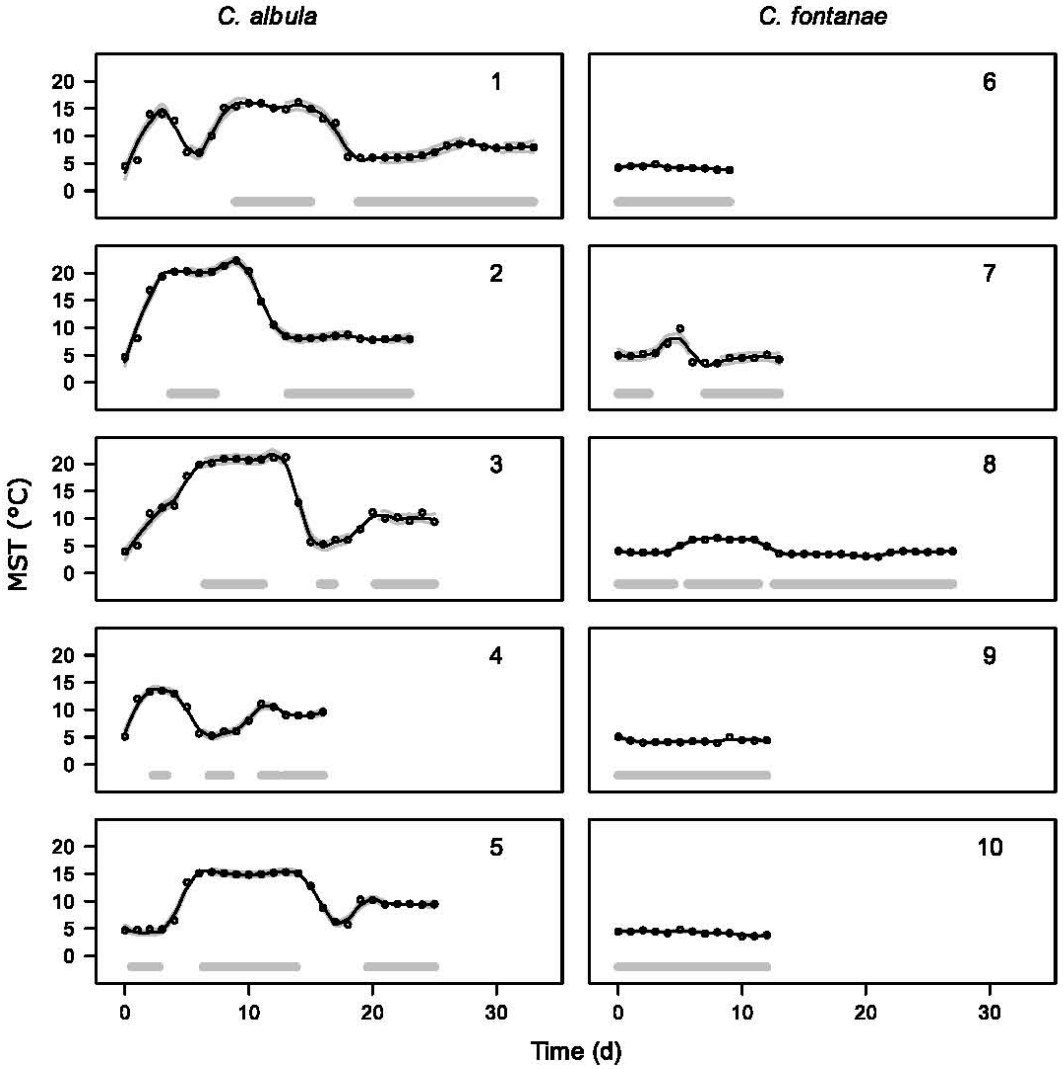

Fig. 1. Time-series of mean selected temperatures (MST, °C) during shuttle-box tests for all groups of vendace (*C. albula*, 1–5) and Fontane cisco (*C. fontanae*, 6–10). Daily mean values (circles) were fitted using a loess smoother (solid lines) and its standard error (dashed lines). An interval sequence approach was used to identify qualitatively similar periods of each time-series. This was done to compare the constant periods of the time-series, as indicated at the bottom of each graph (grey bars), within and between species.

below 5°C after a few days. Qualitatively, a significantly higher proportion of the vendace groups (5 vs. 1) showed clear indications of overcompensation – that is, a rapid temperature increase at the beginning of the experiment (Fisher's exact test, $P = 0.048$).

The final thermal preferendum (FTP) was calculated as the mean value of the last 5 days of behavioural thermoregulation, since these days were determined as the minimal constant final period in all groups tested. For Fontane cisco, the FTP for all groups ranged from 3.9 to 4.6°C with a species average of 4.2°C, whereas for vendace, the FTP for all groups varied

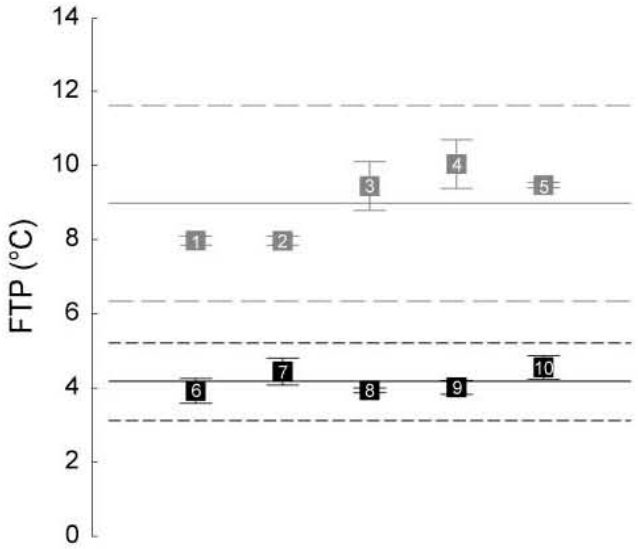

Fig. 2. Final thermal preferenda (FTP, °C) of vendace (*C. albula*, grey) and Fontane cisco (*C. fontanae*, black). Shown are all five groups (numbers according to Fig. 1) from each species (squares) with standard deviation (error bars) as well as the species' grand means (solid lines) with 95% confidence intervals (dashed lines).

from 8.0 to 10.0°C with a species average of 9.0°C (Fig. 2). The difference between the species was significant according to the Welch *t*-test ($t = 10.78$, $df = 4.8$; $P = 0.0002$). Furthermore, the variance of the mean FTP values was significantly lower in Fontane cisco than in vendace groups ($F = 9.87$, $df_1 = df_2 = 4$; $P = 0.048$).

The analysis of metabolic characteristics (data in Ohlberger *et al.*, 2008) revealed different patterns of temperature dependence. Standard and active metabolic rates as well as total costs of transport increased continuously with increasing temperature in both species. In contrast, the net cost of transport, which is the minimum amount of energy needed by a fish to swim one unit distance, was lowest in Fontane cisco at 4°C but lowest in vendace at 8°C (Fig. 3). Temperature effects were significant in both species according to *F*-statistics ($F > 8$, $df = 26$; $P < 0.01$).

DISCUSSION

The two coregonids from Lake Stechlin showed clearly different temperature selection behaviours during the course of the experiments. Vendace initially preferred relatively high temperatures and finally gravitated to intermediate temperatures around 8–10°C, whereas Fontane cisco generally selected low temperatures around 4°C. Consequently, the species had significantly different final preferenda with an average species-specific FTP of 9.0°C for vendace and 4.2°C for Fontane cisco. The determined FTPs correspond to optimum temperatures of net swimming costs in both species.

The temperature preference experiments in this study were performed for at least 2 weeks, although it is generally believed that fish gravitate to their final thermal preferenda in electronic shuttle-boxes within 1–2 days (McCauley, 1977). Vendace selected significantly higher temperatures during the first days than they finally preferred. We thus have to conclude that

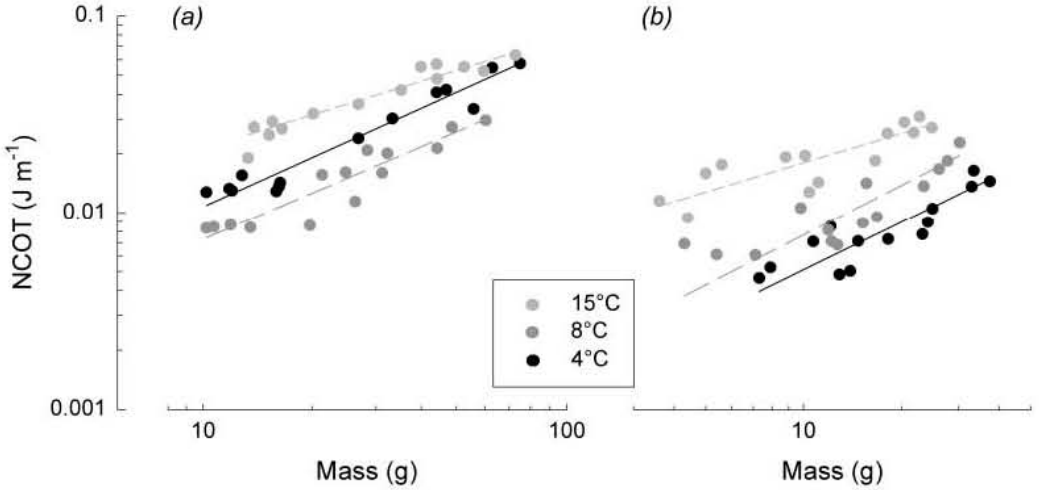

Fig. 3. Net costs of transport (NCOT, J·m^{-1}) for (a) vendace (*C. albula*) and (b) Fontane cisco (*C. fontanae*) at 4°C (black, solid line), 8°C (dark grey, long-dashed line), and 15°C (light grey, short-dashed line) as a function of body mass (g) according to a two-parameter power function (NCOT = $a \cdot M^b$). NCOT represents the minimum energy needed by a fish to swim one unit distance. The mass range differed according to the size difference of the Fontane cisco and vendace populations. The original data are available in Ohlberger *et al.* (2008) and methodological details can be found in Ohlberger *et al.* (2007). Note: double-logarithmic plots.

previous preference tests may have failed to determine FTPs in fish due to short test periods. The initially high-temperature preference of vendace may be explained by over-regulation as an adaptive strategy after acclimation to sub-optimal temperatures (Huey and Berrigan, 1996). Ectotherms generally experience a decelerating effect of cold temperature on cellular processes. In fishes, cold acclimation increases muscle aerobic capacity through an increase in mitochondrial volume density or changes in mitochondrial oxidative capacity (Sänger, 1993; Guderley and Johnston, 1996). Thus, as opposed to evolutionary cold adaptation, short-term acclimation to lower temperatures causes a rise in mitochondrial density or enzyme concentration, which increases maintenance costs (Pörtner, 2002; Guderley, 2004). This cellular mechanism may cause the initial shift of vendace to above-optimum temperatures after cold acclimation to re-establish lower mitochondrial maintenance costs via a reduced mitochondrial density. Fontane cisco, in contrast, may not experience elevated energy costs due to evolutionary cold adaptation. However, our explanation for this compensatory mechanism remains speculative and demands further research.

Our conclusion is supported by the fact that the observed difference in final thermal preference is generally consistent with a previous study on the species' metabolic rates (Ohlberger *et al.*, 2008). Respiration analyses revealed significant differences in temperature-dependent metabolic costs indicating that Fontane cisco are competitively superior at lower temperatures in terms of metabolism (i.e. they experience lower metabolic costs), whereas vendace experience lower energy costs at higher temperatures. Additionally, our recent analysis on swimming costs indicates that the species' net costs of transport are lowest in Fontane cisco at 4°C and in vendace at 8°C, which is in good accordance with the species-specific final thermal preferenda of 4.2 and 9°C respectively. Hence, swimming performance is optimized at the respective thermal preference of both species.

The simultaneous evolution of divergent temperature-dependent swimming costs and the divergence in thermal preferences that correspond to temperatures maximizing swimming efficiency (Figs. 2 and 3) indicate that thermal preference and at least some thermal performance functions, like the net costs of transport, are co-adapted in these fish. The close correspondence is in line with the results of other studies on the physiology of ectotherms that concluded that temperature preference is adaptive (e.g. Johnson and Kelsch, 1998) and usually corresponds to body temperatures that maximize performance (Angilletta *et al.*, 2002, 2006; Beitinger and Fitzpatrick, 1979). However, it has to be mentioned that the thermal preference of fishes is influenced by various factors such as season (Tapaninen *et al.*, 1998), photoperiod (Staaks, 1996), ontogeny (Edsall, 1999), salinity (Stauffer and Boltz, 1994), oxygen concentration (Schurmann *et al.*, 1991), and nutritional status (van Dijk *et al.*, 2002). Hence, preferred temperatures may reflect species-specific values only to some degree, and the concept may not hold for comparisons when studying species with highly variable thermal preferences, such as those observed for instance in anuran amphibians (reviewed in Navas *et al.*, 2008). Nevertheless, the concept has been confirmed repeatedly in fish studies and thermal gradients are widely used as a comparative method to detect interspecific differences between species (Coutant, 1977; Konecki *et al.*, 1995; Larsson, 2005).

Previous ecological surveys on the Lake Stechlin coregonids have revealed a spatial segregation along the vertical gradient of the lake (Helland *et al.*, 2007). The Fontane cisco population inhabited deeper and cooler water layers than vendace and was never found in water layers with a temperature above 7.7°C (Helland *et al.*, 2007). However, the observed FTP of Fontane cisco is remarkably low, since the general thermal optimum for coregonines, including the genera *Stenodus*, *Prosopium*, and *Coregonus*, ranges from 8 to 15°C (Elliott, 1981). The FTPs of *Coregonus* spp. have rarely been analysed. The only final preferendum test on vendace comes from Tapaninen *et al.* (1998), who studied populations from deep Finnish lakes and reported a thermal preference of 9°C for immature fish after long-term acclimation to 2°C. Thus, the calculated FTP for vendace from this study is in good accordance with data in the literature, whereas the FTP for Fontane cisco is far below all values reported to date. The comparison further supports the conclusion that a character shift in thermal preference primarily occurred in Fontane cisco due to adaptation to cooler temperature rather than in vendace.

Comparisons between closely related sympatric species like that performed in the present study provide the most convincing evidence for the importance of temperature adaptation in ecological and possibly evolutionary diversification on small spatial scales. Ecologists have recognized that gradients in water temperature can create spatial structures in fish distribution (Rudstam and Magnuson, 1985) and the existence of temperature gradients is believed to directly influence the physiologically based competitive strengths of fish populations (Jensen *et al.*, 2006). The temperature regime of a deep temperate lake exerts especially strong selection pressures on pelagic fish populations due to the wide range of water temperatures. In other words, it offers the opportunity for an ecologically based physiological segregation along the temperature–depth gradient, which reduces interspecific competition and thereby facilitates co-existence. An example of adaptive differences in thermal physiology promoting ecological divergence is described for the fine-scale pattern of vertical zonation among closely related intertidal animals (Somero, 2002). Similarly, niche segregation between different salmonid species in Scandinavian lakes is facilitated by differentiation in thermal preference (Larsson, 2005). Ecological segregation by using different microhabitats along the depth gradient of a lake is further believed to facilitate co-existence

of native whitefish (*Coregonus lavaretus*) and invading vendace in the sub-arctic Pasvik hydrosystem (Bøhn *et al.*, 2008).

The reported differences in thermal preference, in combination with temperature-related metabolic adaptations and the observed vertical segregation, suggest environmental temperature as a driving force during the evolutionary divergence of the Lake Stechlin coregonids. Similar processes of ecologically based speciation along steep biotic or abiotic gradients may have occurred also in other freshwater systems, like that suggested for the speciation of the Great Lakes ciscoes (Smith and Todd, 1984). This idea is corroborated by theoretical studies indicating that speciation along environmental gradients may be a common process in nature (e.g. Doebeli and Dieckmann, 2003). The conclusion that the depth of the lake and the corresponding temperature gradient were important prerequisites for the evolutionary diversification is further supported by biogeographical considerations. Sympatric pairs of *Coregonus* spp. occur in only two lakes in Germany, Lake Stechlin and Lake Breiter Luzin (Schulz *et al.*, 2006). These two lakes are the deepest in that region (Mehner *et al.*, 2005) and both are located at the southern border of the distribution range of vendace (Kottelat and Freyhof, 2007). Consequently, we can assume that these lakes have the broadest temperature ranges and the steepest gradients compared with other lakes where vendace occur. This supports our conclusion that the temperature–depth gradient has facilitated an ecological and finally evolutionary diversification.

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Paper V

V

A data-based model of evolutionary fish diversification through thermal adaptation along a temperature-depth gradient

Manuscript

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Manuscript

A data-based model of evolutionary fish diversification through thermal adaptation along a temperature-depth gradient

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ABSTRACT

Theoretical models suggest that sympatric speciation along environmental gradients might be common in nature. Here we present the first data-based model of sympatric speciation in fishes along an environmental gradient. The sympatric speciation of common vendace *Coregonus albula* and Fontane cisco *Coregonus fontanae* in a deep German freshwater lake has been suggested based on genetic analyses. Within this lake, the two species segregate vertically and show metabolic adaptations to, as well as behavioural preferences for, correspondingly different temperatures. We test the hypothesis that this speciation process has been driven by adaptations to different thermal microhabitats along the lake's temperature-depth gradient. Using an adaptive dynamics model with gradual evolution of a quantitative trait describing optimal foraging temperature, we show that under the specific ecological conditions encountered in the lake a hypothetical ancestral population would have encountered a fitness minimum at which selection was disruptive and where evolutionary branching may therefore have occurred. We also show that the resultant evolutionary diversification yields two stably coexisting populations with trait values and depth distributions that are in good agreement with those observed in the lake. We therefore conclude that the ecological conditions in the lake are prone to speciation in these fishes, thus supporting the hypothesis that divergent thermal adaptations along the lake's temperature-depth gradient might have brought about the two species observed today.

Keywords: adaptive dynamics, coregonid fish, evolutionary diversification, sympatric speciation, temperature adaptation

INTRODUCTION

Understanding the emergence of biological diversity by adaptive diversification based on natural selection is a major interest in evolutionary biology. The theory of adaptive speciation suggests that macro-evolutionary phenomena of diversification are ultimately the outcome of micro-evolutionary processes driven by natural selection (Orr & Smith 1998; Coyne & Orr 2004; Dieckmann et al. 2004). Hence, phenotypic and ecological diversity are likely to imply disruptive natural selection arising from differential resource use, competitive intraspecific interaction, and ecological opportunity. Competitive interactions between individuals and populations within the same geographical area, involving the exploitation of different resources or habitats, are thus of major importance for understanding biological diversification caused by frequency-dependent selection. Empirical studies have confirmed that intraspecific competition can be frequency-dependent (Swanson et al. 2003; Schluter 2003). Frequency dependence occurs whenever selection pressures depend on the phenotypic composition of a population, which is a direct, and often inevitable, consequence of the environment a population experiences.

The geographical conditions underlying diversifying processes have long been a focus of debate. However, mechanisms of ecologically based sympatric, parapatric, and allopatric speciation appear to be similar, with disruptive natural selection as the driving force of diversification and the evolution of reproductive isolation as a consequence of divergent selection on traits across environments (Schluter 2000). The process of ecologically based adaptive speciation does not only necessitate the emergence of reproductive isolation during diversification, but it also requires the ability of the incipient species to coexist persistently (Coyne & Orr 2004). Hence, it is often the same ecological conditions that produce disruptive natural selection, cause adaptive speciation through gradual divergent evolution, and enable the coexistence of closely related species in sympatry. Empirical work suggests that competition-driven divergent resource or habitat use causes ecological diversification (Svanbäck & Bolnick 2007) and promotes sympatric speciation (e.g., Gislason et al. 1999; Knudsen 2006) within natural populations.

The theory of adaptive dynamics (Dieckmann & Law 1996; Geritz et al. 1998) has facilitated the construction of theoretical models investigating ecologically based

processes of evolutionary diversification (Doebeli & Dieckmann 2005). This framework allows evaluating the potential for evolutionary diversification in complex systems with emphasis on the ecological conditions promoting selection pressures on specific adaptive traits of natural populations. Frequency-dependent selection on a slowly evolving quantitative trait of an asexually reproducing population leads to evolution in the direction of the local fitness gradient until an 'evolutionarily singular strategy' is reached. This singular strategy can either be a fitness maximum, and hence be evolutionarily stable for a single morph, or it can be a fitness minimum, and hence be an 'evolutionary branching point' leading to the splitting and subsequent divergence of two genetically distinct populations. Adaptive dynamics models have repeatedly shown that natural systems can evolve to such fitness minima where evolutionary branching may occur based on frequency-dependent disruptive selection (for a review see Dieckmann et al. 2004). In most of the existing models of adaptive speciation, ecological specialisation through resource or habitat partitioning is the key driver of evolutionary diversification.

One conclusion from advanced adaptive speciation models is that sympatric speciation is theoretically plausible and may thus be a common process in nature (e.g., Dieckmann & Doebeli 1999). However, theoretical speciation models are not easily evaluated in terms of biological plausibility and may lack ecological realism in their assumptions about system properties. For instance, the parameter region in which branching may occur is only indirectly comparable between models, and quantitative estimates are difficult to make without referring to a particular natural system. Empirically motivated and data-based speciation models including ecologically derived quantitative assumptions are therefore needed to evaluate the importance of these processes in nature. If such specific models are integrated within a common theoretical framework, they might be applicable to other natural systems as well.

One of the best model systems for studying adaptive diversification are temperate freshwater fish occupying postglacial environments in which several taxa exhibit ecological diversity consistent with processes of adaptive speciation (Schluter 2000). There is increasing evidence that ecological opportunity in species-poor postglacial lakes, in combination with high intraspecific competition, promotes adaptive divergence in fishes, for instance in coregonids (reviewed in Hudson et al. 2007). Biotic and abiotic

environmental factors in these systems are potential causes of diversification. Their characteristics determine the strength of selection acting on phenotypic evolution and thus ultimately determine the potential for speciation.

In the deep postglacial Lake Stechlin in Germany, a pair of closely related coregonids coexists within the pelagic area: common vendace (*Coregonus albula* (L.)) and endemic dwarf-sized Fontane cisco (*Coregonus fontanae* (Schulz & Freyhof)). The two species are easily distinguished by differential spawning times and exhibit distinct morphological characteristics (Schulz & Freyhof 2003). Their sympatric evolution has been suggested based on mtDNA and microsatellite analyses (Schulz et al. 2006) as well as recent AFLPs (K. Pohlmann, T. Mehner, unpublished data). The species differ in their average night-time population depths, with Fontane cisco being found a few meters deeper in the water column than vendace throughout the year (Helland et al. 2007; Mehner et al. 2007). This difference in depth distribution is associated with a difference in mean experienced water temperature, which declines continuously with depth (Mehner et al. 2005). Despite the depth segregation, diet compositions of the species are rather similar, with a clear dominance of planktonic food (Helland et al. 2008). Hence, mechanisms reducing competition and thus potentially driving divergence between the species are not related to diet. One mechanism promoting ecological divergence between the species is a difference in temperature-specific metabolic costs (Ohlberger et al. 2008a). This directly influences the competitive abilities of the two populations via their foraging efficiency at a specific temperature. Furthermore, the temperature preferences of the two species differ accordingly and correspond to temperatures of minimal net costs of swimming (Ohlberger et al., submitted), which emphasises temperature as the predominant environmental factor shaping the divergence between the two coregonids. The lake itself features typical characteristics of newly colonised postglacial systems. Intraspecific competition for food resources is high due to the low productivity of this meso-oligotrophic lake, and ecological opportunity is high due to a weak competition with planktivores from other taxa and a low predation risk by piscivores in the deep pelagic area. This setting offers the unique opportunity to investigate whether the observed conditions allow for ecological and evolutionary diversification of pelagic fish populations along the depth gradient of the lake.

The main purpose of this study is to provide an empirically motivated and data-based model for a sympatric speciation process along an environmental gradient. The model aims to describe the diversification of a single ancestral fish population into a species pair by adaptation to different thermal microhabitats along a temperature-depth gradient in a temperate freshwater lake. We evaluate the evolutionary diversification using pairwise invasibility analysis. We thereby examine whether the ecological conditions in our study lake allow for the evolution of an ancestral fish population toward a fitness minimum and the subsequent evolutionary branching into two stably coexisting populations.

METHODS

Model description

We model asexual fish populations along a temperature-depth gradient competing for the same zooplankton resource with a depth-dependent carrying capacity. The populations can adapt to different environmental temperature conditions, i.e. to different locations along the depth axis (x), by evolution on a one-dimensional quantitative trait, which is the temperature-dependent metabolic optimum or optimum foraging temperature T_i. For the evolutionary dynamics, we assume asexual reproduction, a low mutation probability μ, and a small standard deviation σ of mutation steps, so that evolution follows the canonical equation of adaptive dynamics (Dieckmann & Law 1996) in conjunction with fitness-based conditions for evolutionary branching (Geritz et al. 1998), as specified in detail below. The population dynamics are deterministic and structured with regard to depth x, with $0 < x < x_{\max}$. The foraging rate $r_i(T(x),T_i)$ of a morph i is assumed to drop with temperature $T(x)$ on both sides around T_i. Exploitative competition for a single unstructured resource, considered as the predominant cause of competition in the system, is logistic and occurs at each depth. The density at which resources become available is given by a depth-dependent carrying capacity density $K(x)$ which declines monotonically with depth. Gain in biomass density $B_i(x)$ of morph i at depth x, which includes fecundity, is proportional to prey catchability, foraging rate, and the logistic competition factor. Biomass loss, which includes mortality, arises from maintenance

costs $m(T(x))$, which monotonically decrease with temperature. Therefore, the per capita growth rates $f_i(x)$, in terms of biomass, are given by the difference between per capita resource intake rates and per capita maintenance costs. We further assume the rapid redistribution of individuals along the depth gradient following foraging dynamics, which can be chosen gradually between random and optimal by varying a parameter α. Individuals keep adjusting their depth according to their potential consumption rate, a potential predation risk and the resource availability. We further define the number of morphs n and the morph index i, with $i = 1,...,n$ for residents and $i = 0$ for a rare mutant. For the evolutionary dynamics we define the invasion fitness of a rare mutant morph i as f_i, its first derivative (or selection gradient) g_i, and its second derivative h_i. Derivatives of the invasion fitness are taken with respect to the mutant trait value and are evaluated at the trait value of the corresponding resident. Appendix A gives an overview of all variables and parameters used in this study and a schematic illustration of the initial model conditions is presented in Fig. 1.

Model dynamics

The population dynamics are described by the change in total biomass of morph i over time,

$$\frac{d}{dt}B_i = f_i B_i,$$

where B_i is the total biomass and f_i is the average growth rate of morph i. The total biomass is given by the integral of biomass density from zero to maximum depth,

$$B_i = \int_0^{x_{max}} B_i(x)\, dx,$$

where $B_i(x)$ is the biomass density of morph i at depth x. The invasion fitness, i.e. the average growth rate at demographic equilibrium is given by

$$f_i = \frac{1}{B_i}\int_0^{x_{max}} f_i(x)B_i(x)dx, \text{ with}$$

$$f_i(x) = \lambda C_i(x,T_i)P_{eq}(x) - \gamma m(T(x)),$$

where $f_i(x)$ is the morph's per capita growth rate at depth x, λ is the conversion factor from mass zooplankton to fish mass, i.e. the energy assimilation efficiency of the fish, and γ converts energy to fish mass. $C_i(x,T_i)$ is the potential consumption rate of morph i with temperature optimum T_i at depth x, $P_{eq}(x)$ is the equilibrium resource density at depth x and $m(T(x))$ reflects the temperature-dependent maintenance costs. We assume a fast resource dynamics so that the zooplankton density is always at equilibrium (see Appendix B for details).

For the adaptive foraging dynamics, we assume that an individual fish with temperature optimum T_i adjusts its depth according to its potential consumption rate ($C_i(x,T_i)$), the equilibrium resource density of the zooplankton ($P_{eq}(x)$), and a foraging probability due to a potential predation risk by piscivorous predators ($F(x)$),

$$B_i(x) = B_i \times \frac{(C_i(x,T_i)P_{eq}(x)F(x))^{\alpha}}{\int_0^{x_{\max}} (C_i(x',T_i)P_{eq}(x')F(x'))^{\alpha}\,dx'},$$

where α is the degree of foraging optimality (with $\alpha = 0$ representing a random and $\alpha \to \infty$ an optimal foraging strategy).

The evolutionary dynamics of the trait value, i.e. the metabolic temperature optimum, is given by the canonical equation of adaptive dynamics, which assumes infinitesimal small individual mutation steps (Dieckmann & Law 1996),

$$\frac{d}{dt}T_i = \frac{1}{2}\mu\sigma^2 \frac{B_i}{b_i} g_i,$$

where μ is the mutation probability, σ^2 is the variance of the phenotypic effect of a mutation, b_i is the average biomass of an individual of morph i, $\frac{B_i}{b_i}$ is the equilibrium population size, and g_i is the first derivative of a mutant's invasion fitness, i.e., its selection gradient.

Parameter estimation

For details on the parameter estimations for the temperature gradient, foraging probability, resource density, maintenance costs and the potential consumption rate

please consider Appendix B and Fig. 2. All these parameters were estimated based on observational data from Lake Stechlin and various laboratory measurements on the two coregonids.

The foraging optimality was estimated based on the distribution of the coregonids in Lake Stechlin. This parameter gives information about the degree to which individuals forage at their temperature optimum and is thus a critical factor for the model dynamics. In order to estimate the degree of foraging optimality in the natural system, we run our model without the evolutionary dynamics but with the empirically determined temperature optima as fixed trait values and compared the resulting centres of gravity with the measured year-round centres of gravity of the Lake Stechlin coregonids (Helland et al. 2007). Fig. 3 shows the cumulative derivation of the two modelled population depths from the natural system as a function of α. The plot suggests that a foraging optimality of about 6 is the best approximation for the foraging behaviour of the fish. We therefore used this α-value for our further analyses.

Evolutionary analysis

We use a pairwise invasibility analysis to investigate the evolutionary dynamics in our system under the specific ecological conditions. This adaptive dynamics tool requires the assumptions that any mutant introduced to the system occurs at very low numbers and that the resident population has reached its equilibrium with respect to size, distribution and trait value at the time the mutant is introduced.

To evaluate the potential for directional evolution on the adaptive trait of an established resident population, that is, the potential for invasion of a mutant, we need to calculate the invasion fitness of a mutant with a slightly different trait value (see Metz et al. 1992). The invasion fitness is given by the sum of the mutant's growth rates at each depth and is always evaluated at demographic equilibrium (see Appendix B). In case of a positive invasion fitness the mutant generically replaces the resident (Geritz et al. 2002) where after a monomorphic population with the new trait value is established. This directional selection on the trait of the monomorphic population proceeds as long as selection drives the evolution in either direction, i.e., as long as the selection gradient

$$g_i = \left.\frac{\partial f_0}{\partial T_0}\right|_{T_0 = T_i}$$

is positive or negative. The point where directional selection comes to an end is referred to as 'evolutionary singular strategy' (Metz et al. 1996). Once evolution has reached the evolutionary singular strategy, selection becomes either stabilizing or disruptive, depending on where in the fitness landscape the singular strategy is situated. In case it is located at a fitness maximum, no invasion of any mutant morph is possible, so that the singular strategy is evolutionary stable and thus considered a 'continuously stable strategy'. If the singular strategy is instead located at a fitness minimum, it is evolutionary unstable, that is, a mutant can invade the system and establish a second resident population. Such a point where selection becomes disruptive, is then called 'evolutionary branching point' (Metz et al. 1996; Geritz et al. 1998). Evolutionary branching occurs if the second derivative of the invasion fitness,

$$h_i = \left.\frac{\partial^2 f_0}{\partial {T_0}^2}\right|_{T_0 = T_i},$$

is positive. A branching point means that a mutant can invade the system, however, for dimorphic evolution to proceed, the two morphs must be able to persistently coexist. Such a protected dimorphism is only possible for trait combinations where a mutant morph can invade the resident morph and vice versa. The direction of the dimorphic evolution after branching is calculated in a manner similar to that for the monomorphic case. We test for invasibility of a mutant into a system with two established residents. In case of a positive invasion fitness for a mutant with a slightly differing trait value, it successfully invades the system and replaces the respective resident. The dimorphic evolution again proceeds as long as disruptive selection drives the populations apart until a point is reached where the selection gradients vanish. At this point, further evolutionary branching may occur if the populations are at a fitness minimum. Otherwise, evolution comes to a halt.

RESULTS

To investigate the evolutionary dynamics of our model and to answer the question whether evolutionary branching may occur under the data-based parameter estimates and assumption in the system with its specific ecological conditions, we apply the above described adaptive dynamics methods and visualize our results using a 'pairwise invasibility plot' (PIP) and a 'trait evolution plot' (TEP).

Fig. 4 shows the PIP for all trait value combinations of two morphs with temperature optima between 4 and 12°C at a foraging optimality α of 6. A monomorphic population starting with any trait value is subjected to directional selection and evolves on the diagonal line until the singular strategy is reached at about 8.5°C. At this point, the population experiences a fitness minimum and selection becomes disruptive so that evolutionary branching occurs. In Fig. 5, we use a TEP to visualize the subset of trait combinations where the two morphs are able to persistently coexist (grey area) as well as the dimorphic evolution after branching (thick dashed lines). The two morphs evolve within the area of coexistence to a point where both selection gradients vanish (black dots). The respective trait combinations are 5.2 and 9.7°C, or, equivalent to this, 9.7 and 5.2°C. At this point the isoclines of the zero selection gradients intersect. On the TEP, these isoclines indicate whether fitness is at a minimum (thin solid lines) or at a maximum (thick solid lines). Since both isoclines are at a fitness maximum at the intersection point, the resulting dimorphism is evolutionary stable. Therefore, no secondary branching can take place in the system and the two morphs are recognised as the evolutionary outcome of our model.

We can now compare the model outputs for the trait values and the biomass distributions with those values observed in the natural system (Fig. 6). Fig. 6a shows the evolution of the trait value, i.e. the optimum foraging temperature, and compares it with the observed temperature preferences of the two coregonids from Lake Stechlin. The temperature optima at the endpoint of the dimorphic evolution (5.2 and 9.7°C) are in good accordance with the measured temperature preferences of the two coregonids (4.2 and 9.0°C, Ohlberger et al., submitted).

Fig. 6b shows the final depth distribution of the two populations after evolution has come to a halt and compares their average depths, or centres of gravity, with the year-round average depths observed for the two coregonids in the lake. Again, the average centres of gravity of the model populations at the endpoint of the dimorphic evolution (17 and 23m) match very well with those observed for the coregonids in Lake Stechlin (17 and 24m, Helland et al. 2007).

The main target parameter for a sensitivity analysis of the model is clearly the foraging optimality, since the evolutionary dynamics are highly dependent on this parameter. An α-value of less than about 1 results in an evolutionary stable strategy at an intermediate temperature optimum, whereas sufficiently large values may lead to a secondary branching event. However, the α parameter was estimated based on the distribution patterns of the coregonids in the lake. Therefore, we have no reason to assume that the value used here over- or underestimates the foraging optimality of the natural population to a degree that would challenge our general evolutionary analysis and conclusions.

DISCUSSION

With the analysis of the model's evolutionary dynamics we have shown that, under the specific conditions of the system, evolutionary branching of a monomorphic population into two populations with distinct trait values and depth distributions may occur. We can thus conclude that the ecological conditions in the lake are prone to evolutionary branching and that sympatric speciation in these fishes is an ecologically plausible scenario. This conclusion supports our earlier hypothesis that the adaptive diversification of the coregonids might have resulted from thermal metabolic adaptation to divergent temperature regimes along the vertical lake axes within the pelagic habitat. Since the speciation process would have occurred along an environmental gradient, i.e. along a spatial dimension in our model, the process might be considered as nearly parapatric. With this model, we present the first description of such a sympatric/parapatric speciation process in fishes along a temperature gradient. It is further the first data-based model of an adaptive diversification along an environmental gradient supporting the idea that this process might be common in nature.

The evolutionary endpoint of the model is a dimorphic fish population consisting of one morph with a lower temperature optimum (5.2°C) occupying deeper water layers (23m) and another morph with a relatively high temperature optimum (9.7°C) occupying shallower water layers (17m). This model outcome matches very well with the depth distributions of the coregonids in Lake Stechlin (Helland et al. 2007) and the trait values found for the two species according to thermal preference tests (Ohlberger et al. submitted) and the respective metabolic optimum temperatures for swimming (Ohlberger et al. 2008a). These consensuses show that the two populations that evolve in our model represent the natural populations very well in their distribution and trait values. A similar situation like observed for Lake Stechlin is found in the nearby lake Breiter Luzin, where a vendace population coexists with a deeper living sister species (*C. lucinensis*) that might have evolved via sympatric speciation (Schulz et al. 2006). Physiological data do not exist for the latter species, but it can be speculated that the ecological conditions, in particular the temperature-depth gradient, in lake Breiter Luzin have produced selection pressures that lead to a similar diversification of the coregonids in that lake.

Environmental gradients are common phenomena in nature with a major effect on biogeographical patterns of species distributions. The biogeography of fishes, for instance, is shaped to a large extent by temperature effects (Pörtner et al. 2007). However, the importance of such environmental gradients for small-scale patterns of species distributions and their diversification has long been a matter of debate. The traditional concept states that gradual evolution on a quantitative trait of a single population along an environmental gradient would necessarily result in local adaptation to an optimum with some smooth and continuous variation around this optimum due to the effects of gene flow and stabilizing selection. Recently, theoretical models have been developed showing that frequency-dependent selection due to competition in trait space and spatial competition for resources can create a discrete clustering of an adaptive trait along continuous environmental gradients. These models demonstrate the plausibility of evolutionary branching in asexual populations (Mizera & Meszéna 2003; Leimar et al. 2008) as well as the evolution of reproductive isolation in sexual populations (Doebeli & Dieckmann 2003) under such ecological conditions. The results presented in this study support the theory of ecologically based adaptive diversifications. The two populations

segregate in space, but within a continuous geographical area, which is commonly referred to as parapatric speciation.

The presented model is liable to some limitations with respect to the conclusions for the speciation process that can be derived from it. The model examines the ecological, physiological and behavioural aspects of an evolutionary diversification without considering the genetic architecture of the process. Hence, evolutionary branching in our asexual model is not equivalent to speciation, since reproductive isolation cannot be directly inferred. However, it signals that adaptive speciation is promoted by natural selection under the specific ecological conditions. In other words, an evolutionary branching in an asexual adaptive dynamics based model implies evolution towards disruptive selection and thus towards a dimorphism within the respective ecological model in which branching is found, thereby indicating that the ecological system under study is prone to speciation (Dieckmann et al. 2004). Further, reproductive isolation between the Lake Stechlin coregonids is attained via separation in spawning time. This separation might have evolved as a by-product of divergence in thermal habitat use due to slower maturation and later spawning at cooler temperatures.

Future work may use the present model as a starting point to develop a predictive evolutionary model that evaluates the impact of future temperature changes due to global warming on the Lake Stechlin coregonids. Besides a changing temperature gradient itself, such an analysis should include changes in the zooplankton distribution and a shift in the thermocline as already predicted for Lake Stechlin (Gerten & Adrian 2001). It would provide insights into the potential impacts of temperature changes on species' interactions and their adaptive responses. These have been largely ignored in the climate change debate so far due to the scarce knowledge in this field.

APPENDIX A

Overview of variables and parameters

Notation	Units	Description
x	m	Depth
x_{max}	m	Maximum depth
$T(x)$	°C	Temperature at depth x
T_{opt}	°C	Temperature optimum
T_i	°C	Temperature optimum of morph i
i	-	Morph index ($i = 1,\ldots,n$: residents; $i = 0$: a rare mutant)
$P(x)$	kg s^{-1} m^{-1}	Zooplankton production at depth x
$P_{eq}(x)$	kg m^{-1}	Equilibrium resource density at depth x
$K(x)$	kg m^{-1}	Carrying capacity of the zooplankton at depth x
r_P	kg s^{-1}	Renewal rate of the zooplankton
λ	-	Conversion factor mass zooplankton to fish mass
$C_i(x,T_i)$	s^{-1} m^{-1}	Potential consumption rate of morph i with T_i at depth x
$r_i(T(x),T_i)$	-	Foraging efficiency of morph i with optimum T_i at $T(x)$
$n_i(x)$	-	Foraging success of morph i at depth x
$m(T(x))$	J s^{-1}	Per capita maintenance costs at temperature T
γ	kg J^{-1}	Conversion factor energy to fish mass
$B_i(x)$	kg m^{-1}	Biomass of morph i at depth x
B_i	kg	Total biomass of morph i
b_i	kg	Average biomass of an individual of morph i
$f_i(x)$	kg s^{-1} m^{-1}	Per capita growth rate of morph i at depth x
f_i	-	Invasion fitness of morph i
g_i	-	First derivative of invasion fitness of morph i
h_i	-	Second derivative of invasion fitness of morph i
μ	-	Mutation probability
σ	-	Standard deviation of mutation steps
α	-	Degree of foraging optimality

APPENDIX B

Temperature gradient

The temperature gradient (Fig. 2a) was derived based on temperature measurements from Lake Stechlin in 2005 (T. Mehner, unpublished data). The function was fitted to the average year-round temperatures according to a Gaussian curve of the form

$$T(x) = 4.5 + 7e^{-\frac{x^{2.5}}{37.5^2}},$$

where 4.5 and 7 represent the asymptotic temperature value at maximum depth and the temperature difference surface to bottom (°C), respectively.

Zooplankton density

The depth-dependent zooplankton densities (Fig. 2b) were fitted to data on the average zooplankton densities in Lake Stechlin from 2005 (Helland et al., 2007; I.P. Helland, unpublished data) according to an exponential function,

$$Z_{obs}(x) = 7e^{-0.04x}.$$

This function was used to estimate the resource carrying capacity (see below).

Foraging probability

The foraging probability function (Fig. 2c) was based on observations in Lake Stechlin showing that the coregonids rarely forage in the epilimnion, although food availability is highest near the surface (Helland et al. 2007). However, predation risk by the main predator in the system, perch (*Perca fluviatilis*), is also higher in the epilimnion (Mehner et al. 2007) and it has been found that this species is almost inactive at temperatures below 10°C (Karas and Thoresson 1992). Moreover, foraging animals commonly seem to alter their behavior according to the spatial structure of both, prey availability and predation risk (see Searle et al. 2008). This is a behavioural trade-off between maximizing both food and safety commonly referred to as the 'landscape of fear'. The foraging probability was thus defined as a rate which decreases from a value of 1 in the colder deeper zones to 0 at 10°C, which is just above the thermocline,

$$F(x) = \delta(2 - 0.2\overline{T}(x)), \text{ with } \delta = 0 \text{ for } T(x) > 10°C.$$

Equilibrium resource density

The depth-specific change in resource density $P(x)$ was calculated according to the following equation,

$$\varepsilon \frac{dP(x)}{dt} = r_P P(x)(1 - \frac{P(x)}{K(x)}) - C(x,T_{opt})B(x)P(x),$$

where $K(x)$ is the carrying capacity density at depth x, r_P is the renewal rate of the zooplankton, and ε is the rate of change in the resource density. $C(x,T_{opt})B(x)$ gives the total resource consumption at depth x, calculated as the sum of the zooplankton consumption of all morphs at depth x,

$$C(x,T_{opt})B(x) = \sum_{i=1}^{n} C_i(x,T_i)B_i(x).$$

The resource dynamics of the zooplankton in the lake are assumed to be much faster than the fish population dynamics ($\varepsilon \to 0$). This leads to a depth-specific equilibrium resource density, which has to be greater than zero,

$$P_{eq}(x) = \frac{r_P - C(x,T_{opt})B(x)}{r_P} K(x), \text{ if } P_{eq}(x) > 0 \text{ and 0 otherwise.}$$

The total carrying capacity of the zooplankton ($K(x)$) was estimated as 5 times the observed zooplankton density ($Z_{obs}(x)$) in the lake,

$$K(x) = 5Z_{obs}(x).$$

The renewal rate (r_P) of the zooplankton was set to be 18% day^{-1} based on trophic transfer efficiencies in Lake Stechlin (Schulz et al. 2004). Similar rates for zooplankton growth have been used in other fish population models as well (e.g., Claessen et al. 2000).

Potential consumption rate

The potential consumption rate of the fish $C_i(x,T_i)$ was calculated according to

$$C_i(x,T_i) = C_{\max} r_i(T(x),T_i)n(x),$$

where C_{max} is the maximum consumption rate of an individual fish under optimal light and temperature conditions, $r_i(T(x),T_i)$ is the foraging efficiency of morph i with temperature optimum T_i at temperature $T(x)$ and $n(x)$ is the foraging success as a function of light at depth x.

The foraging efficiency (Fig. 2d) was estimated based on data on the temperature-dependence of the coregonids' swimming performance. It was fit to the temperature-dependent costs of transport in vendace (Ohlberger et al. 2007) according to a Gaussian function with the assumption that foraging rate drops symmetrically around the optimum temperature,

$$r_i(T(x),T_i) = 0.2 + 0.8e^{-0.05(T(x)-T_i)^2}.$$

The light dependency of the foraging success (Fig. 2e) was estimated based on measurements of the feeding efficiency of the Lake Stechlin coregonids at different light levels (Ohlberger et al. 2008b). According to these data, the decrease in foraging success with decreasing light intensity was best represented by a simple exponential relationship,

$$n(x) = e^{-0.05x}.$$

Maintenance costs

The maintenance costs (Fig. 2f) were described as a function of temperature and biomass according to the energetic models by Ohlberger et al. (2007),

$$m(T(x)) = 0.82B_{mean}^{0.93}e^{0.07T(x)},$$

where B_{mean} is the average biomass of an individual.

Invasion fitness of a mutant morph

Since any mutant invading a system with n resident morphs is infinitesimal rare, it is assumed to have an infinitesimal small biomass, whereas the residents are assumed to be at demographic equilibrium. Hence, the growth rate at each depth of a mutant morph can be calculated based on the biomasses and consumption rates of the residents at equilibrium,

$$f_0(x) = C_0(x,T_0)\left(1 - \frac{C(x,T_{opt})B(x)}{r_P}\right)K(x) - m(T_0(x)), \text{ with}$$

$$C(x,T_{opt})B(x) = \sum_{i=1}^{n} C_i(x,T_i)B_i(x).$$

The invasion fitness of the mutant is given by

$$f_0 = \int_0^{x_{max}} f_0(x)H_0(x)dx,$$

where $H_m(x)$ is the mutant's depth distribution depending on its potential consumption rate, the potential predation risk and the equilibrium resource distribution, which in turn only depends on the resident biomass distributions of the resident morphs,

$$H_0(x) = \frac{(C_0(x,T_0)P_{eq}(x)F(x))^{\alpha}}{\int_0^{x_{max}} (C_0(x,T_0)P_{eq}(x)F(x))^{\alpha}\,dx}.$$

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FIGURE CAPTIONS

Fig. 1: Schematic illustration of the Lake Stechlin system. The coregonids inhabit the open-water area of the lake below the thermocline, i.e. the hypolimnion, where they segregate ecologically and physiologically along the vertical temperature-depth axis. Zooplankton density declines continuously with depth. The highest carrying capacity of the resource is thus found close to the surface where primary production is largest as well. In this area above the thermocline there is a potential predation risk due to perch that forage in the epilimnion.

Fig. 2: Data-based input parameter functions (solid lines) and directly measured data points (open circles) (please consider the Appendix B for details): a) Temperature as a function of depth [$T(x) = 4.5 + 7e^{-\frac{x^{2.5}}{37.5^2}}$]; b) Zooplankton density as a function of depth [$P(x) = 7e^{-0.04x}$]; c) Foraging probability as a function of depth [$F(x) = \delta(2 - 0.\overline{2}T(x))$, with $\delta = 0$ for $T(x) > 10°C$]); d) Foraging success as a function of depth [$n(x) = e^{-0.05x}$]; e) Foraging efficiency as a function of temperature [$r_i(T(x), T_i) = 0.2 + 0.8e^{-0.05(T(x)-T_i)^2}$]; and f) Maintenance costs as a function of temperature [$m(T(x)) = 0.82B_{mean}{}^{0.93}e^{0.07T(x)}$].

Fig. 3: Determination of the foraging optimality alpha (α). Shown is the mean deviation (m) between the centres of gravity of the modelled compared to the natural populations as a function of α. The foraging optimality is a measure of the degree to which individuals of a population forage according to their physiological optimum, the potential consumption rate and the potential predation risk. The α-value with the least deviation (α = 6) was used for further analyses.

Fig. 4: Pairwise invasibility plot (PIP) for trait value combinations between 4 and 12°C at α = 6. The monomorphic population continuously evolves on the diagonal line until a singular strategy is reached at about 8.5°C, which is convergence stable. At this point, it experiences a fitness minimum and selection becomes disruptive. The grey areas indicate at which trait value combinations a mutant phenotype can invade a resident phenotype, i.e. where the mutant invasion fitness is positive.

Fig. 5: Trait evolution plot (TEP) showing the area of coexistence as well as the dimorphic evolution after branching (α = 6). The grey areas represent trait value combinations at which the two morphs can stably coexist, i.e. where a protected dimorphism is possible. Arrows indicate the direction of the selection gradient within the coexistence area. The evolutionary dynamics after branching (dashed line) converge to a protected dimorphism (black dots). Since the intersection point of the isoclines (solid lines) is located on a fitness maximum (thick solid lines), the resulting dimorphism is evolutionary stable. The two established residents have the trait values 5.2 and 9.7°C.

Fig. 6: Comparison of model outcome vs. natural system: a) Evolution of trait values over time (solid and dashed lines) in comparison with the traits of the natural populations (dotted lines). The outcome for the optimum foraging temperature of the dimorphic evolution (5.2 and 9.7°C) is in good accordance with the measured temperature preferences of the two coregonids (4.2 and 9.0°C, see Ohlberger et al. submitted); b) Corresponding final depth distribution at the evolutionary endpoint (solid and dashed lines) with calculated centres of gravity for the model populations (solid and dashed horizontal lines) in comparison with the average centres of gravity of the Lake Stechlin coregonids (dotted lines). The mean population depths are similar in the model outcome (17 and 23m) and the natural system (17 and 24m, Helland et al. 2007).

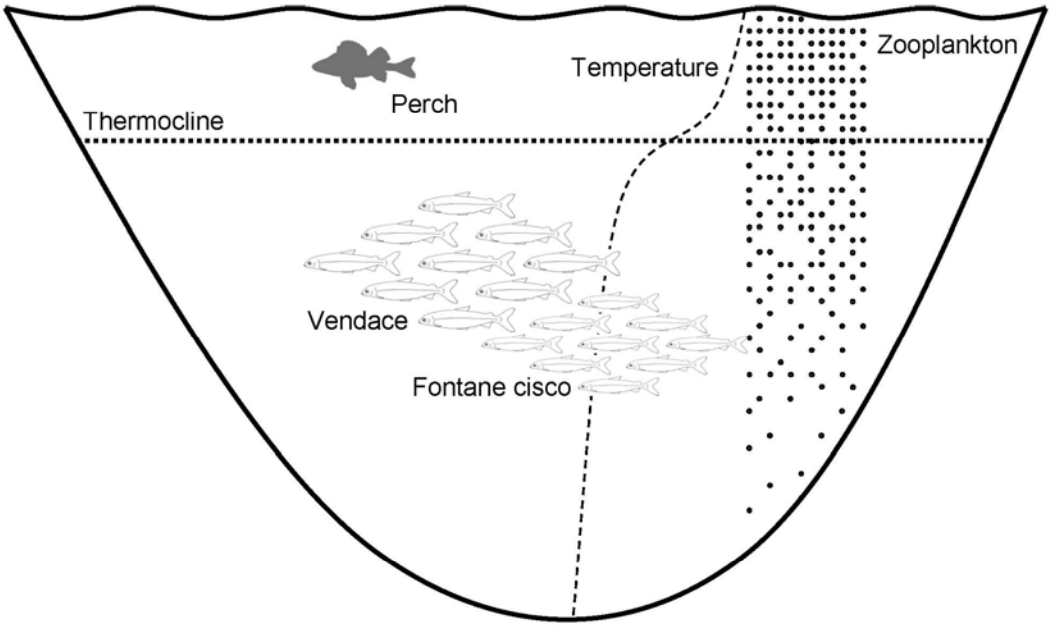

Zooplankton
Temperature
Perch
Thermocline
Vendace
Fontane cisco

Fig. 1

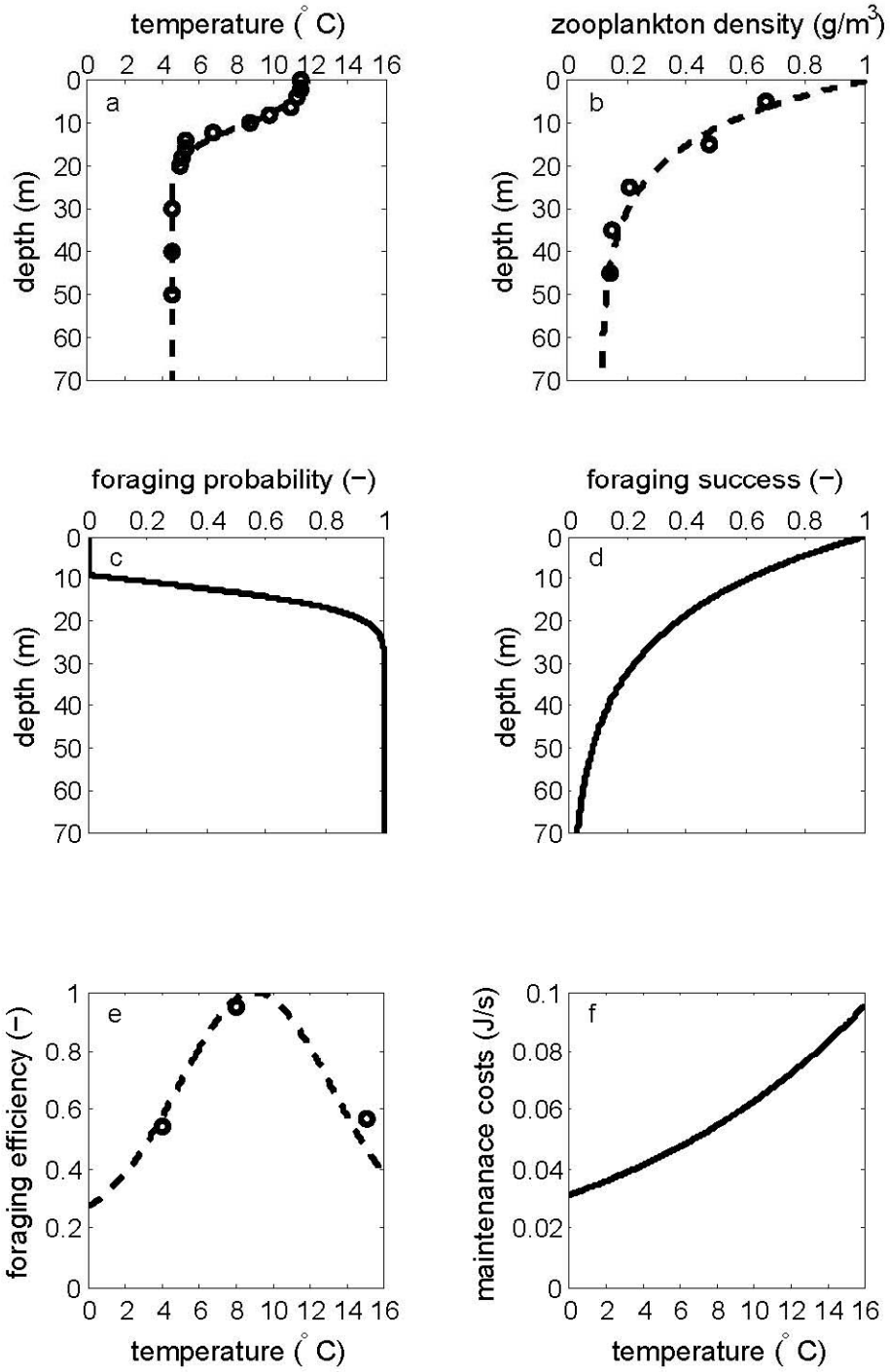

temperature (° C)
zooplankton density (g/m3)
depth (m)
a
b
foraging probability (−)
foraging success (−)
c
d
foraging efficiency (−)
maintenanace costs (J/s)
e
f
temperature (° C)
temperature (° C)

Fig. 2

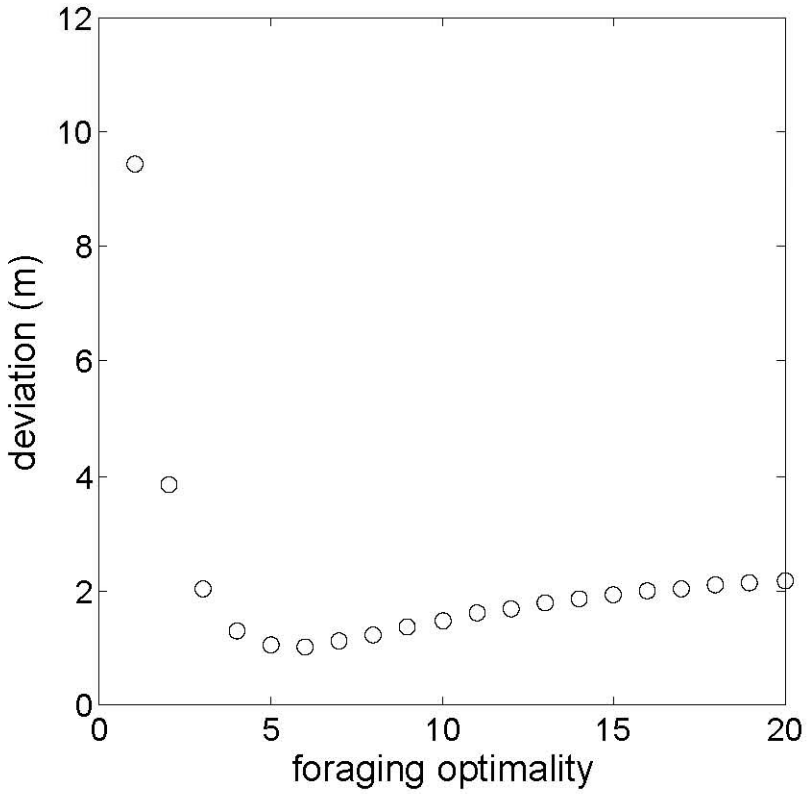


Fig. 3

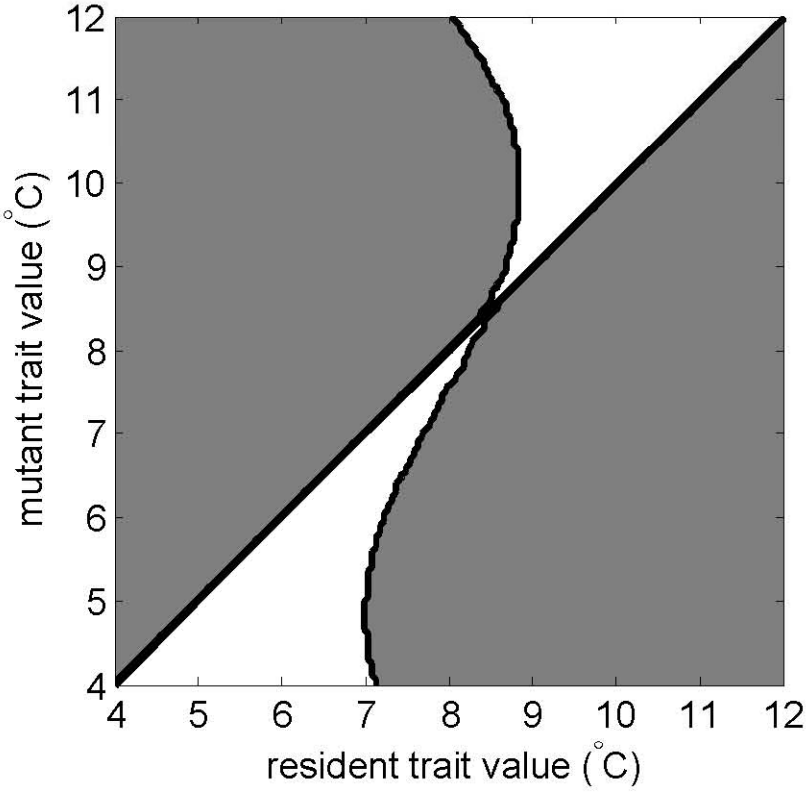

12
11
10
9
8
7
6
5
4
mutant trait value (°C)
4
5
6
7
8
9
10
11
12
resident trait value (°C)

Fig. 4

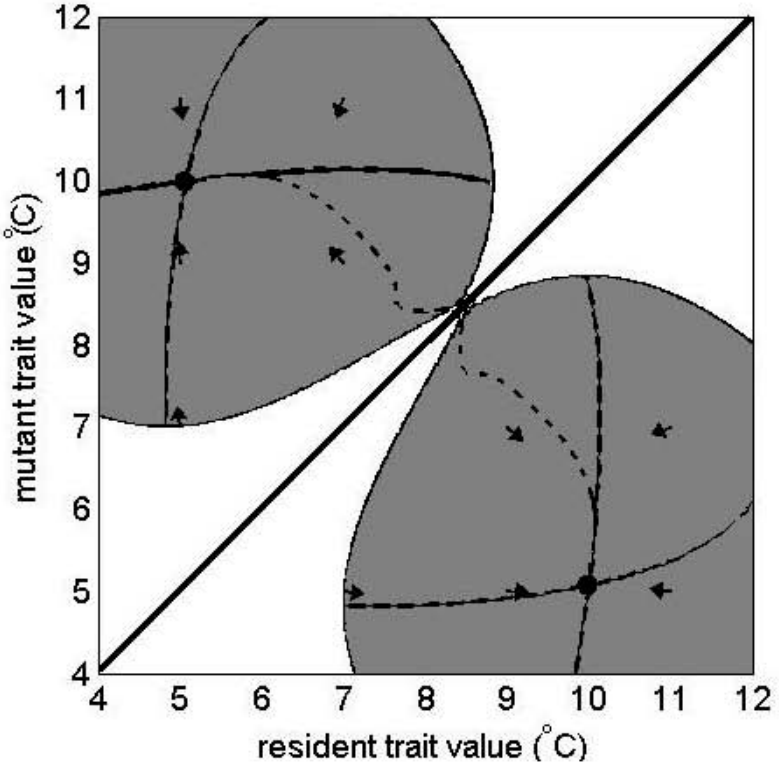
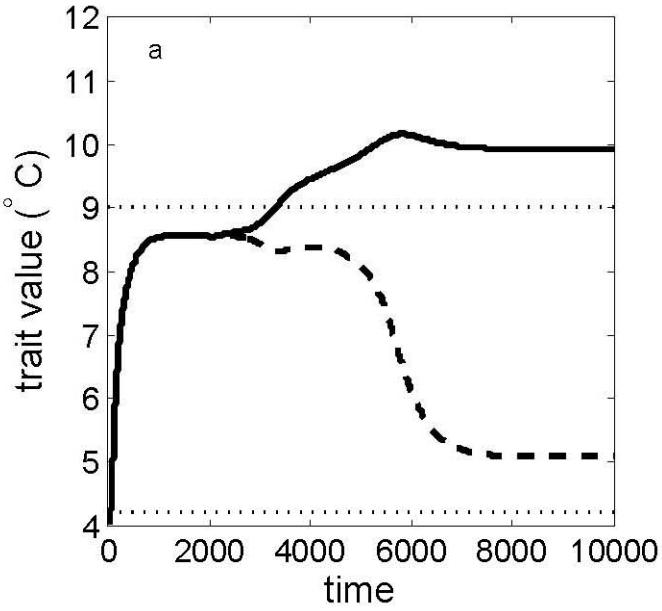

12
11
10
9
8
7
6
5
4
mutant trait value (°C)
4
5
6
7
8
9
10
11
12
resident trait value (°C)

Fig. 5


a
trait value (°C)
time
12
11
10
9
8
7
6
5
4
0
2000
4000
6000
8000
10000

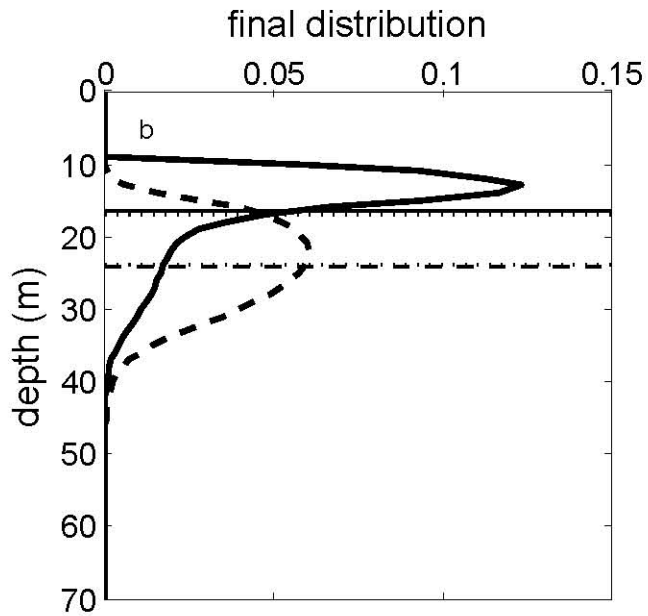

final distribution
b
depth (m)
0
0.05
0.1
0.15
10
20
30
40
50
60
70

Fig. 6

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